DIAGNOSTIC EFFICACY OF THIN PREP PREPARATION (LIQUID BASED CYTOLOGY) IN COMPARISON TO CONVENTIONAL PAP SMEARS AS A PRIMARY SCREENING TOOL FOR CERVICAL LESIONS

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CERTIFICATE

This is to certify that this dissertation **"DIAGNOSTIC EFFICACY OF THIN PREP PREPARATION (LIQUID BASED CYTOLOGY) IN COMPARISON TO CONVENTIONAL PAP SMEARS AS A PRIMARY SCREENING TOOL FOR CERVICAL LESIONS"** is a bonafide work done by **Dr. PREMALATHA. A** in partial fulfillment of the award of **M.D. Degree in Pathology (Branch- III)** under my guidance and supervision during the academic year 2012-2015.

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I **Dr.PREMALATHA. A.** here by submit the dissertation titled **"DIAGNOSTIC EFFICACY OF THIN PREP PREPARATION (LIQUID BASED CYTOLOGY) IN COMPARISON TO CONVENTIONAL PAP SMEARS AS A PRIMARY SCREENING TOOL FOR CERVICAL LESIONS"** done in partial fulfilment for the award of the degree **M.D Pathology (Branch- III)** in Sree Mookambika Institute of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of **Dr. ELIZABETH CHACKO, MD.**

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DISSERTATION SUBMITTED FOR THE DEGREE OF

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1

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"A human being without the milk of human heart is an exquisite rose without fragrance"

"The journey which begins with the sun shine on the morning dews will always glitter" My journey which has now attained its final countdown would not be complete if the following people are not acknowledged.

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To conclude I would like to quote

“Once you replace negative thoughts with positive ones,

you will start having positive results”

-Willie Nelson

CONTENTS

ABSTRACT

TITLE: DIAGNOSTIC EFFICACY OF THIN PREP PREPARATION (LIQUID BASED CYTOLOGY) IN COMPARISON TO CONVENTIONAL PAP SMEARS AS A PRIMARY SCREENING TOOL FOR CERVICAL LESIONS.

Introduction- Cancer of the uterine cervix is the leading cause of mortality and morbidity among women throughout the world. It is the second most common malignancy among women globally and third largest cause of cancer mortality in India after cancers of the mouth, oropharynx and oesophagus.

Aims and Objectives- To study the changes in the cervical cytology smears in the age group of women between 25-60 years, and to compare the efficacy of liquid based cytology smears with conventional pap smears.

Materials and methods - This is a cross sectional study done on 110 women from the OPD of Obstetrics and Gynecology. Conventional and liquid based cytology smears were taken from them. The cytology smears were reported according to the 2001 Bethesda system.

Results- In our study, maximum number of participants 65(59.1%) were in the age group of 41-50yrs and 13(11.8%) had history of chronic vaginal infection. Among the 110 conventional pap smears, unsatisfactory smears were 6 (5.5%), and there were no unsatisfactory smears in liquid based cytology. The most common finding in cervical smear cytology was inflammatory smears, followed by normal and atrophic smear. Abnormal epithelial lesions were detected more in liquid based cytology.

Conclusion- It is concluded that thin prep liquid based cytology modality shows more intraepithelial lesions than conventional smears.

Key words- Cytology, conventional pap smear, liquid based cytology smear.

Cancer of the uterine cervix is the leading cause of mortality and morbidity among women throughout the world[1]. It is the second most common malignancy among women globally[2]. Nearly 4 lakh new cases are diagnosed annually and 80% of deaths occur in developing countries[3]. India has got high burden of cervical cancer and accounts for one fifth of the world burden of cervical cancer[4].

Cervical cancer is the third largest cause of cancer mortality in India after cancers of the mouth, oropharynx and oesophagus[5]. It accounts for nearly 12% of all cancer related deaths in the country[6]. Women of low socioeconomic status, low educational level, early marriage, multiple pregnancies, prolonged use of oral contraceptives, harmones, smoking, genital infections with herpes, Human papilloma virus are at higher risk of developing cervical cancer. (Shanta et al 2000)[7] . The mortality of cervical cancer is expected to 474,000 women per year by 2030[8].

HPV infection is highly associated in sexually active women. Among several types of HPV, 16 and 18 is strongly associated with cervical cancer[9]. For the development of precursor lesions and cervical cancer persistent HPV infection is the risk factor. Integration of HPV DNA into host cervical cells

is essential for the development of cervical cancer. This leads to disruption of the E2 gene. They induce increased expression of HPV derived oncoproteins (E6 and E7),with subsequent inactivation of p53 and retinoblastoma (Rb) tumor suppressor proteins, which results in unregulated cellular proliferation through release of transcription factor E2F.This leads to increase in p16 protein expression a biomarker in the identification of cervical intra epithelial lesions[10.]

Immunological factors like type-1 cytokines, interleukins-2 (IL-2) interferon (INF-γ) are immunostimulatory and are capable of limiting tumor growth. The type-2 cytokines, IL-4, IL-10 are immunoinhibitory and are capable of stimulating tumor growth. The cytokine network is disrupted by HPV infection. Immunocompramised women infected with HIV are at increased risk of developing cervical intraepithelial neoplasia. Extensive HPV infection is associated with a pronounced shift from type 1 to type 2 cytokines[11].

Cervical cancer are common in women aged 30 – 59 years, with the peak age for incidence varying with populations[12]. The majority are detected in late stages due to lack of information. The incidence of cervical cancer is reduced by cervical screening. So early detection of precursor lesion in the cervix can be done by cytological screening by doing a pap smear. It is a

simple, safe and non invasive method and is widely accepted screening technique[13].

According to the American College of Obstetrics and Gynecology effective cervical cancer prevention is to identify the precursor lesions and to provide appropriate treatment to women. Precursor lesions have the capacity to progress to invasive cervical cancer if left untreated[14].

The sensitivity in conventional pap smear is reduced to less than 50% due to poor quality of sampling and preparation, presence of obscuring blood, inflammation and overlapping epithelial cells[15]. This gave rise to false positive and false negative test results[16]. To overcome these shortcomings advanced newer technology liquid based cytology has been introduced in the 1990s. LBC improves sensitivity and specificity and reduce the unsatisfactory smears. HPV-DNA test is performed with the residual material[17].

Cervical cancer is potentially preventable and effective screening programmes reduces the mortality in many developed countries. This happens due to the detection of precancerous lesions. Cervical cancer when detected at the earliest stage , the 5 year survival rate is approximately 92%[18].

Newer vaccines have been approved for preventing cervical cancer. Two vaccines Cervarix™ (GlaxoSmithKline) and Gardasil™ (Merck and Co) are very effective in the prevention of persistent HPV infection with HPV 16 and 18[19].Treatment of cervical cancer depends on the stage of the cancer and other prognostic factors. The main type of treatment includes surgery, cone biopsy, hysterectomy, radiation therapy and chemotherapy.

Since early detection predicts better prognosis, one of the most effective way of preventing and controlling cervical cancer is regular screening and early diagnosis[20]. Cervical cancer if detected at an early stage is preventable and curable [21].

Ever since the discovery of effectiveness of pap smear in detecting epithelial changes in uterine cervix prior to progression to carcinoma cervix, pap smear has been widely used as a mass screening method[22].

Aims and objectives

1. To study the changes in cervical cytology smears of women in the age group of 25 – 60 years.
2. To compare the diagnostic efficacy of liquid based cytology smears with conventional pap smears.

Review of literature

Cervical cancer is defined as:

It is a proliferation of malignant cells that arise in cervical tissue and represents a continuum of conditions ranging from non-invasive to invasive carcinoma, commonest is squamous cell carcinoma[23].

Screening :

A screening test should be cost effective, less invasive, easy to perform and highly sensitive that can be applied to a large number of apparently healthy individuals. The success of screening programme is directly related to the method used, available financial resources and influenced by the patients cultural and educational profile**.**

The advantage of cervical cancer **s**creening is done to identify the early stage of cervical cancer thereby further progression of the disease and the mortality can thus be reduced[6]. The main objective of the National Cervical Cancer screening programme is to detect and treat the precancerous lesions. It thereby reduces the incidence and mortality[24]. There are various screening procedures.

Methods of cervical screening:

- Conventional exfoliative cytology
- Liquid based cytology
- Automated cervical screening techniques
- Visual inspection of acetic acid (VIA)
- Visual inspection of lugols iodine (VILI)
- Speculoscopy
- Cervicography
- HPV DNA testing
- Colposcopy
- Fluorescence spectroscopy
- Polar probe
- Among these the best method of screening available is the cervical cytology screening[25].

Newer screening Technologies[26.:]

- Liquid based cytology
- HPV DNA

Newer techniques that employ liquid-based cytology have been developed to improve the sensitivity of screening. The reduction in inadequate rates from using LBC is of considerable benefit to women.

Both conventional and liquid based methods are acceptable for screening.

Screening intervals[27]**:**

Age to initiate screening:

Three years after the onset of sexual activity, not later than the age of 21 years.

Screening frequency:

Annually with conventional cytology or every two years with liquid based cytology. After the age of 30 years, women with three consecutive normal tests may be screened every 2-3 years.

Screening after hysterectomy:

No cytological testing after total hysterectomy for benign conditions.

Discontinuation:

After the age of 70 years.

Routine screening for HPV infection:

Not yet FDA approved conventional or liquid based cytology combined with the test for DNA from high risk HPV types should be

performed not more often than every 3 years. The 20th century achieved a remarkable decline in the mortality of cervical cancer in developed countries due to the implementation of Pap Test[28]. This achievement is directly attributable to the implementation of pap test.

History of Pap Test :

The microscopic appearance of cells from the vagina was illustrated by observers Donne and Beale. In 1847, a Frenchman, F.A. Pouchet , published a book dedicated to the microscopic study of vaginal secretions during the menstrual cycle. In the closing years of the 19th century, illustrations of cancer cells derived from cancer of the uterine cervix were published. In 1927, C. Daniel, reported that cervical smears, obtained by means of a bacteriologic loop, fixed with methanol and stained with Giemsa, were an accurate and reliable method of diagnosing cancer of the uterine cervix. In 1928, Babes published an extensive beautifully illustrated article on his subject in the French publication, Presse Medicale[29]. He introduced cytological sampling of the uterine cervix for the diagnosis of cancer.

Pap smear is considered to be the most effective and successful test in cancer reduction programme. Credit for its conception goes to George N. Papanicolaou, the father of cytopathology. In the year 1924, George N Papanicolaou, an investigator interested in the endocrinology of the

menstrual cycle made an incidental observation that the cancer cells are derived from the uterine cervix may be observed in the human vaginal smears. He presented this observation in May 1928. Papanicolaou also identified cancer cells in sputum, urine, gastric washings and breast secretions[30] .

In 1939, Papanicolaou entered into an association with the Cornell Gynecologist Herbert Traut, and worked to prove his idea. In the vaginal pool smears provided by Traut, Papanicolaou identified cancer cells in a number of patients with malignant tumors of the uterine cervix and endometrium. In 1941, both published their findings as 'The Diagnostic Value of Vaginal Smears in Carcinoma of the Uterus," in the American journal of Obstetrics and Gynecology. In 1943, this was followed by a monograph , "Diagnosis of Uterine Cancer by the Vaginal Smear". In 1947, a Canadian Gynecologist , J. Ernest Ayre documented taking samples directly from the cervix with a wooden spatula was more efficient rather than a vaginal smear taken with a pipette., In 1956, he published landmark Atlas of Exfoliative Cytology[30] .

Exfoliative Cytology:

Exfoliative Cytology is based on spontaneous shedding of cells derived from the lining of an organ into a body cavity. They can be removed by non abrasive techniques . Shedding of cells is a phenomenon based on constant renewal of an organ's epithelial lining. The age of these cells cannot be determined within the sample. Vaginal smear prepared from cells removed from the posterior fornix of the vagina is the typical example.

Evolution of Bethesda system:

Various Classifications and Terminologies in cytology :

PAPANICOLAOU CLASSES:

The terminology developed by Papanicolaou separated cervical cytological findings is to five categories or classes.

The original Papanicolaou classification came in 1954.[31]

Class Description:

1. Absence of atypical or abnormal cells
2. Atypical cytology, but no evidence for malignancy
3. Cytology suggestive of, but not conclusive for malignancy
4. Cytology strongly suggestive of malignancy
5. Cytology conclusive for malignancy

It lacks equivalent terminologies for histopathologically diagnosed lesions and does not mention about non neoplastic conditions. Then WHO terminology was introduced.

WORLD HEALTH ORGANIZATION TERMINOLOGY[32]:

In 1950-The WHO terminology allows more precise correlation between cytological and histopathological findings (Riotten et al 1973). These are

1. Mild dysplasia
2. Moderate dysplasia
3. Severe dysplasia
4. Epidermoid carcinoma in situ
5. Epidermoid carcinoma in situ with minimal stromal invasion
6. Invasive epidermoid microcarcinoma
7. Invasive epidermoid carcinoma.

But there are many disadvantages in WHO terminologies. They showed higher rates of intra-observer and inter-observer variation with cervical cytology. Non neoplastic conditions and specimen adequacy are not adequately dealed with the WHO terminology. (Sherman ME et al 2001).

For clear understanding of the cervical neoplasia pathogenesis, the cervical intraepithelial neoplasia (CIN) terminology was introduced in the late 1960s (Richart 1973) .

CERVICAL INTRAEPITHELIAL NEOPLASIA[32]

(CIN)TERMINOLOGY

The CIN concept emphasizes that dysplasia and carcinoma in situ represents different stages of the same biological process. It had a major impact on how precancerous lesions are treated, since all types of cervical cancer precursor were considered to form a biological and clinical continuum. The CIN terminology includes

1. CIN 1
2. CIN 2
3. CIN 3

The CIN terminology is still widely used in many countries for reporting both histological and cytological diagnoses. As a result of various terminologies, National Cancer Institute convened a workshop of expert consultants, cytopathologists and representatives from other organizations to review the existing terminology and to recommend effective methods of reporting.

2001 Bethesda system:

The workshop held at the US National Institutes of Health conference in Bethesda, Maryland in 1988[32]. A new terminology was developed to provide better standardization and uniform reporting of pap smears. This terminology is known as The Bethesda System (TBS). On the basis of experience obtained during the first three years of its use, re- evaluation and revision in responses to advances is needed for the clinicians and cytopathologists. In 1991 the Bethesda System was slightly modified to consider areas for possible improvement .After the invent of role of HPV in the pathogenesis of cervical neoplasia TBS was once again revised. Additionally, algorithms for the treatment and follow-up of intraepithelial lesions were inconsistent. As a result, the third workshop was held in April 30-May2, 2001, with more than 400 participants. The old system was updated and it was further modified and The Bethesda System 2001 was developed. (Solomon et al 2002) [33.]

It reflects the pap test abnormalities and addresses new screening technologies.

The Bethesda System-2001 comprises of multiple components[34].

The overall structure of the TBS 2001 reporting system is similar to the previous system (TBS 1991) but there are several important changes. That includes

1. The adequacy statement of ‘‘satisfactory but limited by’’ has been dropped. The Pap test is now interpreted either as satisfactory or unsatisfactory for evaluation and not further classified according to a limitation.
2. The report is considered to be an ‘‘interpretation’’. It is not given as a diagnosis.
3. All negative Pap tests are reported as ‘‘negative for intraepithelial lesion or malignancy,’’ or ‘‘NILM.’’ under the general interpretation. This term may be compared with the finding of organisms, reactive changes, atrophy and other benign findings. In contrast to the previous 1991 Bethesda system, whereby ‘‘within normal limits’’ /Benign cellular changes was reported alone.
4. ‘Infection’ categories are changed to ‘Organism’.
5. The reporting of benign reactive changes is optional. Documentation of reactive changes in the report to spot trends in a series of cervical cytology specimen from same patient. Some studies showed a mild increase in the incidence of SIL in cases

interpreted as reactive compared to that reported as within normal limits.

6. Ancillary testing and automated review can be done.

Classification of various terminologies[32]:

Table – 1 Classification of various terminologies

Modified Papanicolaou	WHO	CIN	Bethesda
Class I			**Within normal limits**
Class II			**Benigncellular changes,ASC**
Class III	**Mild dysplasia**	**CIN I**	**LGSIL**
Class III	**Moderate dysplasic**	**CIN II**	**LGSIL**
Class III	**Severe dysplasic**	**CIN III**	**HGSIL**
Class IV	**Carcinoma insitu**	**CIN III**	
Class V	**Microvasive carcinoma**	**Invasive carcinoma**	**Invasive carcinoma**

Gross anatomy of the cervix:

Cervix is the narrow caudal portion of the uterus. It is conical in shape with a truncated apex directed downwards and backwards. It measures 2.5cm and is continous with the body of the uterus and below it protrudes into the vagina forming fornices. The four fornices are anterior, posterior, and two lateral. The posterior fornix is deeper than the anterior. The portion between the cervix and corpus is called isthumus**.** The cervix is divided into two portions. The portio vaginalis which is that part protruding into the vagina and the portio supravaginalis, which lies above the vagina and below the corpus. The portiovaginalis is covered by non keratinizing squamous epithelium. It canal is lined by a columnar mucus secreting epithelium which is thrown into a serious of V-shaped folds that appear like the leaves of a palm and are therefore called plicae palmitae. The upper border of the cervical canal is marked by internal os, where the narrow cervical os widens out into the endometrial cavity .The lower border of the canal, external os, contains the transition from squamous epithelium of the portovaginalis to the columnar epithelium of the endocervical canal. This occurs at a variable level reliable to the os and changes with hormonal variations that occur during a woman life. It is in this active area of cellular transition that the cervix is most susceptible to malignant transformation[35].

The original squamous epithelium of the vagina and cervix has four layers.

Basal layer (Stratum germinatum):

It rests on the basement membrane. It consists of a single row of cuboidal or columnar cells with scanty basophilic cytoplasm, and centrally placed round to oval large nucleus.

Parabasal or prickle cell layer:

It is above the basal layer.4-10 cells in thickness consisting of large polyhedral cells with basophilic cytoplasm and centrally placed nucleus, arranged in an irregular mosaic pattern.

Intermediate cell layer:

It forms the bulk of the epithelium. It is also called clear cell layer. The cells are large oval to polygonal, with irregular vesicular nuclei and give a characteristic basket weave pattern. The cytoplasm is rich in glycogen.

Superficial layer or stratum corneum:

It is made up of flattened, elongated or polygonal cells with acidophilic cytoplasm and small pyknotic nuclei. The cells detached from the surface (exfoliation)[36].

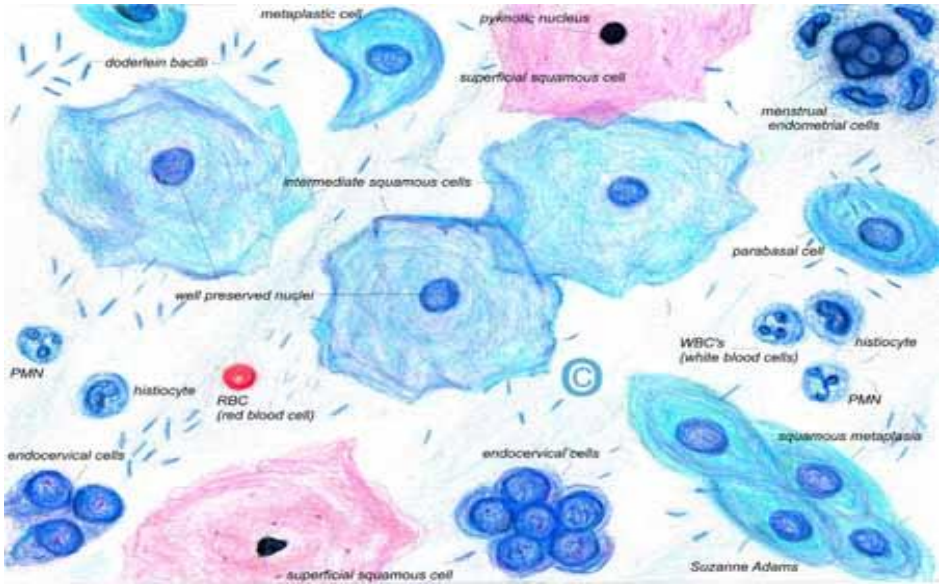
Histology of squamocolumnar Junction:

The original squamo columnar junction represents the junction between the columnar epithelium and original squamous epithelium laid down during the intrauterine life. It is designated as transformation zone in a normal quiescent cervix .The anatomic location of squamo columnar junction in relation to external os is variable considerably with age. Other factors include hormonal status, birth trauma, OC pills use and physiological conditions such as pregnancy[37]. At menopause it is located within the endocervical canal. Transformation zone is the commonest site for precancerous lesions[38,39.]

Normal cytology of Cervix[41]:

Cell originating in the stratified squamous epithelium have been divided into three types. Superficial, intermediate and parabasal. From the deepest layers of epithelium, basal cells are derived and are never seen in smear practically. A maturation index is expressed as a ratio of different types of squamous cells (parabasal: intermediate: superficial). The epithelial cells are classified based on cell size, shape, cytoplasmic staining property and N:C ratio.

Figure – 1 Contents of normal cervical cytology



Superficial squamous cells:

Superficial squamous cells are large polygonal cells measuring 35 – 45µm . The cytoplasm is flat, delicate, transparent and eosinophilic .The size and shape varies. The nucleus is very dense and pyknotic about 4 µm is diameter. The superficial epithelial cells are usually discrete and are not found in groups. The cytoplasm is rich in keratin filaments of high molecular weight, which accounts for the relatively constant polygonal shape of these cells and their resistance to manipulation . The superficial cells originate from the uppermost layer of the stratified squamous epithelium. The

classical features are best seen during the oestrogenic phase of menstrual cycle[41].

Anucleated squames:

Anucleated superficial squamous cells or squames may also occur. They are polygonal. The nuclear zone appear lighter area (nuclear ghost). Because of excessive keratinisation of the cytoplasm which is thick and stains yellow or pale orange. A small,clear central area often marks the site of the nucleus, and cytoplasm is sometimes folded..When nucleated squames are numerous,one must consider the possibility of excessive keratinisation of the cervicovaginal epithelium, which appears clinically as white patches (leukoplakia). Anucleated squamous cells may also occur in keratinising squamous cell carcinomas[42]

Intermediate squamous cells:

Intermediate squamous cells are smaller than superficial squamous cells and are larger than parabasal cells. They are folded and the size varies with a diameter of 30 – 60 μm, have basophilic cytoplasm. The nucleus is open (vesicular) and round to oval measure about 8 μm in diameter. Nuclear membrane is well defined. The nuclear chromatin is fine granular allowing easy identification of chromocenters and sex chromatin. The cells lie in tight groups or discretely in the smear depending upon the hormonal stimulation.

A variant of the intermediate, boat shaped navicular cells occur that store glycogen are seen in pregnancy and early menopouse. Increase of progesterons in pregnancy causes accumulation of glycogen and it is stored in the cytoplasm of intermediate cells[42]. Usually the smear consists entirely of intermediate squamous cells by the 4th or 5th month of pregnancy .

Superficial and intermediate cells may form squamous pearls in which cells are concentrically arranged due to exfoliation of a compressed agglomerisation of squamous cells originating from the bottom of the epithelial pit.The benign squamous pearls have no diagnostic signifance so long as the nuclei are of normal size and shape. Somewhat similar squamous pearls with nuclear abnormalities may be observed in keratinizing squamous carcinoma[41].

Cockleburrs are hematoidin crystal arrays that are surrounded by histiocytes. Their greatest dimension vary up to 100 μm . They are rare in non pregnant women and are more commonly found in pregnant women [43].

Cytolysis or lysis due to fragility of the cytoplasm occurs as a result of fermentation of glycogen by lactobacilli. The intermediate squamous cells are rich in cytoplasmic glycogen, they are the principal targets of the bacteria and destruction occurs by the action of Doderlein bacilli, Trichomonas.

Parabasal cells:

Parabasal squamous cells usually occur singly and are oval or spherical in shape, measure about 12 to 30 μm in diameter. The nuclei of parabasal cells are centrally placed basophilic, open, vesicular. Cytoplasm is usually cyanophilic and sharply demarcated. Exposure to air and dryness may cause cytoplasmic eosinophilia.Occasional nucleoli seen. Immature cells lie in sheets, while mature parabasal cells lie separately. Parabasal cells are predominantly seen in smears of postmenopausal women. In young woman they are seen in an infectious conditions or traumatic process, which results in the loss of superficial layer of the squamous epithelium.

Basal cells are never seen in smear and are small round / oval cells with scanty basophilic cytoplasm. Their nuclear morphology is similar to parabasal cells[41].

Endocervical cells:

Endocervical cells are rarely seen in vaginal smears. They are common in cervical smears obtained by brushing or scraping. Endocervical cells are mucin producing columnar cells with a measurement of about 20 - 30 μm is length and 8-12 μm in diameter. The nucleus is eccentrically placed with fine granular chromatin texture and abundant vacuolated cytoplasm. One or more small nucleoli sometimes are present. They are

larger than endometrial cells, and when the cells are flattened (squashed) along their long axis, they form cluster of polygonal cells with an appearance often described as honeycomb(arranged in sheets) / picket fence (arranged in strips). In the event of inflammation or injury, multinucleated endocervical cells are seen[41].

Columnar endocervical cells encountered in the smear depends on

- Collection ,instrument (spatula, cytobrush)
- Positive of the squamocoloumnar junction, area of metaplasia
- Harmonal contraceptives and in pregnancy-less columnar endocervical cells are seen[42]

Morphology of the endocervical cells depends on the phase of the menstrual cycle. In the proliferative (preovulatory) phase, the cytoplasm of the endocervical cells in sheets is opaque and scanty and the nuclei are closely packed together. In the secretory (postovulatory) phase of the cycle, the cytoplasm is distended with clear mucus, the nuclei show degeneration and in cell sheets, are separated from each other by areas of clear cytoplasm. Some of the columnar cells have a ciliated border.When such cells are numerous they originate in an area of tubal metaplasia.The ciliated portion of the cell may be detached and form a ciliated body (ciliocytophthoria)[41].

Endometrial cells:

In cervicovaginal smears, normal endometrial cells are seen with decreasing frequency from the first to tenth or rarely, twelth day of the menstrual cycle. After twelth day of the cycle or after menopause the presence of endometrial cells are considered abnormal. Endometrial cells originate from the superficial or deep layer of endometrium. Their size vary according to the phase of the menstrual cycle. Endometrial cells occur as balls or single cells[41].

Present as 3-D clusters of cells in liquid based preparation. They have a single nucleus and a prominent granular chromatic pattern and scant cytoplasm. Exfoliated endometrial cells have nuclear moulding and fragmentation. Abraded endometrial cells appear as large/small tissue fragments with glands and stroma. Endometrial cells with a double contour and a core of stromal cells surrounding glandular cells during the first half of the menstrual cycle-Exodus[44]

According to TBS 2001 endometrial cells differ from endocervical cells by nuclear size and scant cytoplasm. Endometrial cells can be found in the smear if IUD has been inserted or hormonal pills are used[41].

Other Cells accompanying epithelial cells in cervicovaginal smears:

Polymorphonuclear leucocytes may be found in small amounts in normal vaginal smear. They are numerous in the second half of the menstrual cycle and during pregnancy, Polymorphonuclear leucocytes have segmented nuclei with two or more dark blue lobes. Cytoplasm contain numerous granules. Small mononuclear macrophages are associated with menstrual flow and early stages of inflammation. When several macrophages fuse, they form giant cells. Such multinucleated cells are observed in atrophic smear and in advanced menopause.[41]

Fibroblasts are young cells of fibrous connective tissue, found in smears of chronic inflammation, trauma, ulcer.

Contaminants:

A large and sometimes exotic variety of contaminants can pollute the smear. Other non epithelial contaminants include spematozoa, lubricating jellies, vaginal cream. or glove powders (talc, starch)[41].

Commensal micro-organisms:

Numerous organisms colonize in the vagina in the absence of disease. They include lactobacillus which appears as blue staining rod shaped organism 1-2μm in length, Diptheroids, Coliforms, Anaerobes and Enterococci.

Menopause:

Menopause is caused by cessation of cyclic ovarian function due to decreased oestrogen activity. This results in the arrest of mentrual bleeding and gradual arrest of maturation of squamous epithelium, loss of supeficial cell layers and replacement by parabasal cells. Because of absence of protective layer and dryness of the epithelium, if offers little resistance to bacterial invasion resulting is vaginitis and cervicitis[46].

Cytological patterns at the time of menopause[41]

Three basic cytological patterns can be differentiated

- Early menopause
- Crowded menopause
- Atrophic or advanced menopause

Early menopause: slight deficiency of oestrogen

The smears are composed of predominantly of dispersed intermediate cells, and some large parabasal cells with a reduction in the proportion of superficial squamous cells. They occasionally show cytolysis.

Crowded menopause: Moderate deficiency of oestrogen

The smears are thick, crowded clusters of parabasal and large intermediate cells are seen. They are similar in size and their nuclei are of

normal sizes. deposits of glycogen in the form of yellow deposits are found in the cytoplasm. These cells are usually well preserved.

Atrophic menopause or advanced menopause

The parabasal cells predominate. In atrophic menopause dryness of the genital tract occurs. Two patterns are observed. A characteristic uniform grey discolouration of the enlarged opaque nuclei with uniform enlargement of parabasal cells. Cytoplasm is markedly eosinophilic and accompanied by nuclear pyknosis or karyorrhexis, denotes the other pattern.

Normal vaginal flora [46]

It is dominated by lactobacilli called bacillus of Doderlein. They are gram positive rods that are immotile and encapsulated. They are connected in chains. They vary in length from 3-6μm.The normal pH of the vagina is maintained by glycogen fermentation, transforming into lactic acid. Normal and intact stratified squamous epithelium is an effective barrier against infection. A vaginal flora predominantly composed of doderlein bacilli can be found in pregnancy, during the second half of menstrual cycle, progesterone containing contraceptives are used.

Squamous metaplasia:

Squamous metaplasia is the normal endocervical epithelium in transformation zone is physiologically replaced by squamous epithelium of

varying degrees of maturity. Electron microscopy studies documented the reserve cells of the endocervical epithelium differentiate either into mucin producing normal endocervical cells. It is considered to be the precursor of squamous metaplasia[47]. It is caused likely by harmones, Oestrogen, chronic inflammation and mechanical pressure as in IUCD.

Immature squamous metaplastic cells can be identified is smear . Cells derived from mature metaplasia resemble normal squamous cells. They are frequently found in tight clusters or groups. Metaplastic cells are composed of angulated cells, with clear borders and cytoplasm may show fine vacuole. Three histological changes in squamous metaplasia [47]

Table – 2 Histological Changes in Squamous Metaplasia

STAGES	CHANGES
STAGE 1	Reserve cell hyperplasia
STAGE 2	Immature squamous metaplasia
STAGE 3	Mature squamous metaplasia

A typical squamous melaplasia:

Minimal dysplasia of immature metaplastic cells is also known as atypical squamous metaplasia, and is characterised by nuclear abnormalities. They show nuclear enlargement or binucleation confined to a few cells

within the cluster. Significant cellular and nuclear enlargement, variability in nuclear size, coarse granulation of chromatin and the presence of prominent nucleoli .

Tubal metaplasia:

The endocervical epithelium show features of tubal metaplasia. It is usually found in hysterectomy specimens. Strips of crowded, columnar and goblet cells with terminal bars and cilia are diagnostic. The nuclei can be enlarged, pleomorphic and hyperchromatic with increased N/C ratio.[49] Peg cells are characterised by dark and granular cytoplasm and elongated nuclei[50].

Changes induced by endocervical brushes:

Reparative changes:

Reparative changes result from injury to the cervical epithelium and the proliferation of reserve cells which grow to reepithelise a focus of ulceration due to previous biopsy, cauterisation. They are characterised morphologically by significant nuclear enlargement and usually the presence of large, prominent nucleoli as a sign of protein synthesis in the fast growing cells. Cells are arranged in flat sheets. The cytoplasm is cyanophilic and sometimes vacuolated. Nuclei are round to oval .Nucleoli are prominent.The nuclear chromatin is finely granular, evenly distributed,

not hyperchromatic, shows mitosis.It is difficult to differentiate between cells from reparative changes and cells from Invasive cancers of low grade adenocarcinoma[43]

Radiation changes:

They are characterized by large bizarre multinucleate cells in groups or in isolation with enlarged nucleus,polychromasia and nucear / cytoplasmic vacuolation .The nuclear cytoplasmic ratio is maintained[43].

Regeneration of cells can occur in squamous epithelium, in squamous metaplastic epithelium and in columnar epithelium.

Cellular changes associated with Intrauterine contraceptive device:

The most reactive change that closely mimic intraepithelial neoplasia, both of squamous as well as glandular type are associated with the presence of IUD[51]. A marked inflammatory reaction is seen. Cells show severe cellular and nuclear pleomorphism an increased N/C ratio, prominent nucleoli and cytoplasmic vacuolization. The cells may be difficult to differentiate from adenocarcinoma[43]. It is necessary to remove the device and repeat the smear after as interval of 4-6 weeks.

Female genital tract and inflammatory processes:

In the female genital tract, inflammation is caused by infections with a variety of microorganisms and parasites or by physical agents (cauterization,irradiation), mechanical factors (trauma) and chemical agents (caustic).

Infection mainly spreads by

- Direct invasion of the genital tract by pathogens,
- Sexually transmitted and
- Blood borne infections.
- Ascending infection causing endometritis, salphingitis [45].

In aute inflammatory process, smears have a dirty appearance, composed of neutrophils, necrotic cells, or cell debris and clumps of bacteria. The squamous cells show moth eaten appearance, cytoplasmic vacuolations. Polymorphs may be seen and nuclei show pyknosis, karyorrhexis, karyolysis, Perinuclear halos and multinucleation are common[52].

Erythrocytes are found in smears when blood has passed through external os. (Menstruation, cervical ruptures, neoplasms), scraping of ectocervix too roughly

Follicular cervicitis:

Benign lymphoid follicles with germinal centers are formed with numerous mitoses. The rupture of these follicles produce a large number of mature and immature lymphocytes[46].

Plasma cell cervicitis

They are characterized by the varying degree of maturity

Infections of the cervix and vagina:

Trichomonas, gardrenella and candida are responsible for 90% of vaginitis.These organisms are considered as risk factors in the etiology of cervical cancer[53.]

Trichomonas infection:

A parasitic protozoan Trichomonas vaginalis causes trichomoniasis. It is a sexually transmitted disease[54]. An increase in vaginal pH and various tissue injuries in the vagina are predisposing factors. The cytoplasm of epithelial cells often shows polychromasia, eosinophilic discolouration and irregular (worm – eaten appearance).

The organism is pear shaped, size varies from 15-30μm, with a pale eccentrically placed nucleus. The cytoplasm contain reddish brown granules, which indicate a phagocytic action. This is commonly accompanied by

leptothrix. A general hypertrophy of the intermediate cells and basal epithelial cells with prominent perinuclear halos are seen[42].

Bacterial Vaginosis:

It is caused by Garderlla vaginalis. Milky Vaginal discharge and a foul fishy odor is noticed. They are seen in women of child bearing age usually associated with intrauterine device or pregnancy[37].

The background of the infected vaginal smear show colonies of a single or multiple bacilli, cocci, living in symbiosis without the expected abundance of inflammatory leuckocytes. Coccobacilli adhere to squamous cells and covered like a carpet (clue cells)[55]. Shift in flora is suggestive of Bacterial vaginosis[56].

Candida:

Fungal organisms candida albicans, candida glaborata are the usual cause of fungal infections.Symptomatic infections occur in patients with diabates mellitus, antibiotics, pregnancy and conditions associated with decreased cell mediated immunity. Pruritis is the most common symptoms with cheesy discharge. Tangles of pseudohyphae admixed with yeast forms are common [37].

Gonorrhea:

Gonorrhoeae, is caused by eosinophilic Gram-negative diplococcus called Neisseria [41]. It can be asymptomatic or manifested by burning sensation and purulent vaginal discharge. The bacteria may be seen within the cytoplasm of neutrophilic polymorphonuclear leukocytes in Papanicolaou- stained smears. Infection is confirmed by bacterial culture for a definite diagnosis.

Actinomycosis:

Actinomyces normally resides in the female genital tract and it does not mean as an indicator of disease. Actinomycosis is characterized by a foul smelling vaginal discharge containing sulfur granules. Large amount of inflammatory leucocytes, histiocytes and necrotic debris are always present. It is commonly caused by Actinomyces israelii in patients with IUDs or vaginal pessaries for contraception, surgical clamps and foreign bodies. These microorganisms are gram-positive and present as irregular, thick bundles or clusters of filaments (Gupta bodies) by Hager and Majmaudar[57].

Herpes simplex virus:

Herpes Simplex virus infection of the genital tract, chiefly caused by HSV-2. The diagnostic infected cells in a vaginal smear are typical and easy to recognize. Cellular changes are characterized by large multinucleated

epithelial cells with moulded nuclei, ground glass appearance and peripheral margination of chromatin. Dense esosinophilic intranuclear inclusions surrounded by halo are seen[57].

Condyloma and HPV – infection:

Condyloma is a sexually transmitted infection caused by HPV[58]. It is commonly diagnosed in younger women (<30yrs) and during the second half of pregnancy. HPV 6 and 11 are commonly associated with low grade dysplasia and HPV 16, 18, 31, 35 are associated with high grade dysplasia and carcinoma in situ. Progression of this HPV infection to cancer may be influenced by other factors including immune suppression, high parity, cigarette smoking and long term use of oral contraceptives[59].It mainly infects immature metaplastic squamous cells present at squamo-columnar junction. But HPV replicates only in the maturing squamous cells.

Koilocytes defined by Koss and Durfee in 1956 are intermediate or superficial cell characterized by an abnormal, enlarged, hyperchromatic, single, double or multiple nuclei surrounded by a large, sharply demarcated perinuclear halo and a residual rim of cytoplasm[60].

Koilocytes must be differentiated from cells with perinuclear halos found in other type of infection, such as trichomoniasis. In these cells, however nuclear abnormalities are not as apparent and halos tend to be smaller and less demarcated.

Immunocytochemical stain can detect viral capsular antigen. Viral capsule formation is a late and focal event. Therefore this staining is positive in only about one half of cases of classic condyloma and mild dysplasia cases. DNA hybridization techniques, by detecting HPV DNA or RNA are more sensitive and specific in diagnosisng HPV infection. Hybridisation techniques can also determine the specific type of HPV details by various DNA techniques[61].

Southern blot which is considered as the 'gold standard' technique for HPV diagnosis. It is a modification of the filter hybridization technique. DNA is extracted from cells and digested with restriction enzymes to cut the DNA at specific sites. The procedure seperates the DNA fragments, using electrophoresis, which are then transferred to a filter support and hybridized with labelled probes[62].

Another method of HPV detection is in situ hybridization, in which a labeled probe is applied to an ordinary tissue section. In contrast with filter techniques, in situ hybridization can be applied to formalin fixed tissue and

allows localization of infected cells. This allows their morphology to be studied,and gives the investigator the ability to retrospectively examine archival specimens..The polymerase chain reaction (PCR),which uses enzymatic DNA amplification is currently the most sensitive method of HPV detection[63].

Inflammation associated cellular changes:

Cytoplasm:

Vacuolization, Perinuclearhalo, Pale stain, Polychromatic, Eosinophilic, Frayed edges, Polymorphonuclear cells.

Nuclearchanges:

Enlargement, Vacuolisation, Chromatin hyperchromatic, bland, Karyopyknosis-rhexis,-lysis.

Background:

Dirty, Inflammatory, Mimics tumor diathesis.

Squamous carcinoma of uterine cervix and its precursor:

From the precursor lesions or abnormal surface epithelium, invasive carcinoma of cervix develops.

Risk factors:[64,65,66,67]**:**

Low economic groups:

There is increased incidence of cervical cancer in women of low socioeconomic status.

Diet and cervical cancer:

Most information available is regarding vitamin A and Vitamin C. Some studies show the inverse relationship between the intake of vitamins and the risk of cervical cancer.

STD:

Epidemiologically cervical cancer behaves like a sexually transmitted disease. It is more common in women who have had multiple sexual partners, who are more promiscuous. It is absent in virgins.

Parity, Cigarette smoking

Smoking is a risk factor independent of sexual parameters, with an overall two fold increase in risk for the development of cervical intraepithelial neoplasia and invasive cervical cancer.

Local Hygiene

Cancer cervix is found to be positively associated with lack of genital washing and negatively with the use of clean sanitary napkins during menstruation.

Long term use of oral contraceptive/IUCD- There is significantly increased risk of cervical cancer in patients who have used oral contraceptives, the incidence increasing with the duration of the use.

Immune deficiencies due to HIV- An increased incidence of cervical intraepithelial neoplasia has been described in patients who are infected with HIV.

Viral agents:

Herpes virus Type 2(HSV–2)-The epidemiological evidence for involvement of Herpes simplex virus in cervical carcinogenesis is suggestive but there is no clear experimental evidence to support it.

Human Papilloma Virus High risk groups HPV 16, 18, 31, 33, 35, 39, 45, 51

HPV 16 is observed in invasive squamous carcinomas and HPV type 18 is observed in small cell carcinoma and adenocarcinomas.

Other factors are young age at first intercourse multiplicity of sexual partners and multiparity

Low grade squamous Intraepithelial lesion (LSIL) [33]:

Low grade squamous intraepithelial lesion (mild dysplasia, CIN grade I). The lesion are generally first observed in young woman or even adolescence. And may be observed in older women, after menopause.The

cells are of superficial and intermediate dyskaryotic cells. Clusters or aggregates of spindly squamous cells with markedly eosinophilic cytoplasm and small pyknotic nucleus, occupying less than one third of the total area of the cell. Nuclear chromatin is finally granular and slightly hyperchromatic. If the population of the dyskaryotic cells is 10% or more of the abnormal cells, it becomes likely low grade lesion, accompanied by a high grade lesion in the adjacent epithelium.

High grade squamous intraepithelial lesion: (CIN II, III)[33]:

Most cases of HSIL develop in endocervical epithelium, either within the transformer zone or in the endocervical canal..It constitutes 0.5% of all pap samples.There are three histologic pattermns of HSIL.-large cell, intermediate, small cell. The lesion is characterized by immature squamous cells. Neoplastic processs is derived from the basal or reserve cells .High grade squamous lesions of metaplastic and small cell type frequently extend to endocervical glands which should not be considered as evidence of invasion. The least frequent histologic pattern is the high grade lesion of squamous type develop in LSIL and progress to HSIL.

High grade lesions may be divided into three principal morphologic groups:

- Keratin forming lesions derived from and retaining the characteristics of squamous epithelium.
- Lesions derived from endocervical epithelium often retaining features of squamous metaplasia.
- Lesions derived from reserve cells, usually of endocervical origin, characterized by small cancer cells.

High grade keratinizing squamous intraepithelial lesions:

The dominant feature is usually the presence of keratin forming cancer cells of a variety of shapes with abundant orange or yellow opaque, thick cytoplasm accounting for the term pleomorphic dysplasia.

Tadpole cells spindly squamous cancer cells may be observed. The nuclei are often pyknotic. Some cancer cells appear as ghosts in which the nucleus has been partially or completely replaced by keratin. Background of the smear shows considerable inflammation.

Intraepithelial lesion with features of squamous metaplasia:

They are characterized by monotonous population of parabasal, dysplastic or cancer cells with marked nuclear abnormalities. The cytoplasm is basophilic.

High grade lesions composed of small cells:

They are characterized by very small cancer cells with scanty, basophilic cytoplasm, owning singly or in clusters. The cells have relatively large, hyperchromatic irregular nuclei. Flattening of edge of cell cluster and whirling in center are suggestive of HSIL over glandular abnoramality.

Microinvasive carcinoma:

Microinvasive carcinoma is the earliest stage in the genesis of invasive cancer. The current definition of microinvasive carcinoma, recommended by the society of Gynaecologic Oncologist is the depth of stromal invasion not greater than 3mm[68].

Cytologic criteria of microinvasion[46]:

1. Occurrence of malignant cells in aggregates or syncytia.
2. Irregular distribution of nuclear chromatin is 50% of cancer cells is seen.
3. Presence of prominent nucleoli.
4. Presence of inflammation and necrosis.

In routine cytodiagnosis, making a firm diagnosis of a microinvasive carcinoma should be avoided.

Invasive squamous cell carcinoma[28]:

Dysplastic cells, including koilocytes

Dedifferentiated and undifferentiated cancer cells, developing marked aberrations in the nucleus and cytoplasm.

Presence of large sheets of cells or fragments of tumour. Naked nuclei are not uncommon. In some cases, the large nuclei of cancer cells may be pale, blend, vesicular and sometimes contain a single visible nucleolus

Atypical squamous cells of undetermined significance (ASC – US):

Atypical squamous cells of undetermined significance is defined according to Bethesda system, as squamous abnormalities that are more marked than those attributable to reactive changes. Also included in the category of ASCUS are lesions that could be considered as normal dysplasia. Criteria are nuclei two an a half to three times the are a of normal intermediate cells nucleus with increased N/C ratio, minimal nuclear hyperchromasia and chromatin irregularity associated with dense orangophilic cytoplasm[42].

Atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H):

It represents 5-10% of all ASC cases. Single cells with a high nucleo-cytoplasmic ratio cells occur singly in small fragments with less than 10

cells. The nucleus are one and a half to two times larger. Nuclear abnormalities like hyperchromasia and irregular chromatin favours HSIL[29].

Atypical Glandular cells[69]:

The classification of glandular abnormalities has been significantly revised in the 2001 Bethesda system

The term atypical glandular cell of undetermined signifance(AGUS) has been eliminated to avoid confusion with ASCUS. It is further subclassified into Atypical glandular cells, endocervical, endometrial or not otherwise specified. Atypical glandular cells favour neoplasia. It has been associated with both benign and malignant lesions of the cervix.

Adenocarcinoma[28]:

Adenocarcinoma constitute 15% of all cancers of the cervix. They are derived from the endocervical epithelium.They have a tendency to exfoliate in small sheets , in loose clusters and in glandular or rosette formation. Their cynanophilic cytoplasm is often abundant, glandular,or lacy with indistinct borders.Their oval nuclei are slightly enlarged with coarsely clumped chromatin with small nucleoli. The background of the smear is usually clean.

Invasive endocervical adenocarcinoma:

The cells are usually columnar, often larger,/smaller than endocervical cells. They form syncytial aggregates, the enlarged nuclei are often eccentric

and are round to oral. They are usually single with multinucleated occurring in 12% of these cells. Loose granular chromatin and multiple nucleoli are present. The nucleolus is prominent and enlarged[42].

Endometrial adenocarcinoma:

It occurs in post menopausal women.usually it is associated with postmenopausal bleeding. Endometroid is the most common type.Cells typically occur singly or in smaller clusters. Cytoplasm is scanty often vacuolated with intracystoplastic neutrophils. Nuclei may be enlarged,vary in size and shape, moderate hyperchromasia, irregular chromatin and parachromatin clearing. Prominent nucleoli seen[28].

A characteristic clinical feature is a watery vaginal discharge, which is seen in the pap smear as finely granular basophilic diasthesis.

Extrauterine adenocrcinoma:

When cells diagnostic of adenocarcinoma occur in association with a clean background or morphology unusual for tumors of uterus or cervix extrauterine sources such as ovary or fallopian tube should be considered. The presence of papillary clusters or psammoma bodies suggests ovarian origin.The lack of tumor diathesis is a clue to non cervical origin [28].

Verrucous squamous cell carcinoma:

It is a rare neoplasia. Infection with HPV is most common.It is a rare variant of squamous cell carcinoma[70]. It includes papillary growth pattern and infiltration with pushing rete peg expansion. It is a low grade cervical intraepithelial neoplasia in a pattern of keratinizing dysplasia.. Few or no koilocytes are seen. Local invasion is common without distant metastasis[71].

Papillary Sqamous cell carcinoma:

Growth patterns are similar to verrous squamous cell carcinoma. They exhibits significant nuclear atypia. Late recurrence and metastasis are common.

Some tumors like small cell carcinoma, glassy cell carcinoma, clear cell carcinoma are rare variants.

Papillary serous adenocarcinoma:

High grade tumor, with prominent nucleoli, numerous papillary groups similar to high grade papillary serous adenocarcinoma of ovary, fallopian tube and peritoneum[72].

Glassy cell carcinoma:

Glassy cell carcinoma is a variant of adenosquamous carcinoma. It is characterized by solid growth of large tumor cells with abundant

eosinophilic cytoplasm. Nuclei are large with prominent nucleoli. The stroma contains an inflammatory infiltrate, often with many eosinophils[73].

Neuroendocrine carcinoma:

The tumor cells are round, with oval nuclei, coarse chromatin and prominent nucleoli.Mitotic figures and tumor diathesis are usually seen[73].

Adenoma malignam:

Rare type of adenocarcinoma, but retains deceptively the normal columnar configuration[73]. Numerous endocervical cells are present in flat, cohesive monolayered sheets and three dimensional clusters. The neoplastic cells vary from small and cuboidal to large and columnar. The nuclei are round or oval and vesicular with fine or coarsely dispersed chromatin.

Clear cell carcinoma:

These tumors grow in young women who have been exposed in utero to di ethylstillbesterol [73].

Conventional cervical cytology:

In Conventional cervical cytology samples are taken from ectocervix & endocervix, and then smearing is done onto glass slides. It is then fixed and stained by Papanicolaou stain.. The Pap smear has been utilized for cervical screening for more than 50 years. It reduces the cervical cancer

incidence to more than 70% [74]. The sensitivity of conventional Pap smears for detection of cervical cancer, precursors was < 50%[15] .

Limitations of conventional pap smear [15,75]:

- Inadequate transfer of cells to slide
- Non representative samples
- In homogenous distribution of abnormal cells,
- Presence of inflammation and blood
- Air drying artifact common
- Background will not be clear
- Overlapping epithelial cells
- Sensitivity is 60-70%
- We cannot perform HPV-DNA testing
- Human error is probably the primary threat to accurate interpretation.

The sensitivity reduces in the amount of blood, inflammation, and thickness of the smear (Sherwani et al2007,Kavatkar et al 2008)

New generation of collection devices has greatly improved the sampling techniques as it dependably removes large and representative samples from the endocervix and ectocervix compared with the conventional smears [76].

To improve the cervical specimen cytology fewer studies have been done and newer technologies came today in practice.

Liquid-Based Cervical Cytology:

It has been approved by the U.S. food and drug administration for primary screening. Automated smear equivalent processing systems, Thinprep and Surepath are used[76]. These new technologies improve the adequacy and reduce false negative rates. It also improves the specificity and sensitivity of the screening.[77] Cytological assessment is better in LBC because of well preserved nuclear details. The cervical cytological sample is dispersed in a liquid suspension. In Liquid based cytology, samples are collected using a cytobrush . Then the tip of the brush containing the sample is rinsed in a vial containing fixative .It yields more representative samples. It is then centrifuged and filtered. Thus a monolayer preparation with well preserved morphology is obtained[78]. These preparations are free of blood and debris, with absence of air drying and obscuring cells and thereby it decreases the number of unsatisfactory slides. An unexpected benefit of liquid based thin layer cytology is identification of presence of infection in the genital tract by molecular testing of the residual material in the vial. The liquid sample further can be used for HPV, DNA testing and other molecular tests.[79]Liquid based cytology reveals the outstanding performance in the

detection of cervical cancer precursors and detection of all abnormalities such as ASCUS[80]. It provides a diagnostic value in malignancies. Liquid based cytology was done on other specimens like endometrium, urine, pleural effusion, breast, thyroid aspirates and ascites.

In cervical cancer screening, liquid-based cytology is an alternative to the conventional Papanicolau (Pap) cytology smear for early detection of cervical abnormalities and cervical cancer.

Liquid based methods add to the cost of a conventional Pap smear. Two types of LBC are in use. The First generation LBC & Second generation LBC[81].

Liquid Based Cytology

First Generation LBC:

Liquid based cytology was introduced in the mid-1990s.Thin prep and Sure path are approved by the FDA. They are used worldwide and for non gynecological cytology smears.

Two technologies [81] :

Thin Prep(1996) and Sure Path(1999).

LBC : Liquid based pap test:

- Employs fluid transport medium to preserve cells.
- Most liquid preservatives.
- Debris are eliminated by automated process.

Thin Prep Method[81]:

In Thin Prep samples are collected by using a FDA approved sampling device cerviex brush. Then the brush is rinsed in a vial containing methanol based fixativc Preserv Cyt solution. The vial is then processed.

In , Thin Prep automated processor, the vial is placed and processing is carried out. During mechanical agitation, clumps of cells and mucus are broken. Liquid based filtration procedure is used, by which the preservative solution is filtered through a membrane filter. The pore size is specifically designed. Red blood cells and inflammatory cells are removed and a representative sample is transferred to a glass slide. This results in a monolayer preparation.

Unique features thin prep pap test slides include uniformity of cell preparation, wet fixation, cell size, smear pattern and specimen background.

Uniformity of cell preparetions:

- Cocentrated
- Evenly distributed
- 20mm circular area

Wet fixation:

- Cells round up in solution
- Enchanced cytoplasmic details
- Methanol based collection solution
- Enchanced nuclear detail
- Variability in chromasia

Cell size:

- Propotionately smaller
- Single cells more prominant

Smear pattern:

- Smear pattern eliminated
- Backround pattern altered

Surepath Method[82]:

In this method samples are collected with a broom like device with a detachable head .Head of the brush is removed from its stem and placed into a vial of ethanol based fixative. This system works on the principle of density gradient .

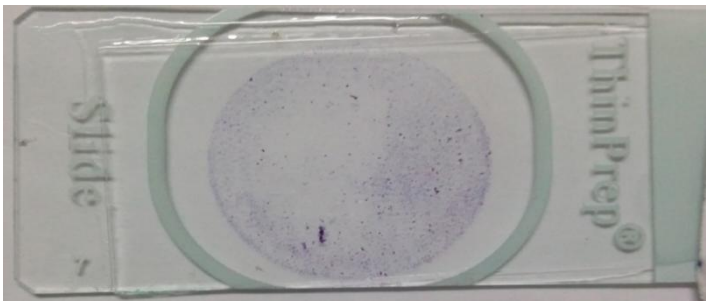
In Sure Path method clumps of cells and mucus are broken up by aspiration through a syringe. The cell suspension is then layered on top of a density gradient and the red blood cells and inflammatory cells are separated

from the epithelial cells by density gradient centrifugation. Then the cell pellet is resuspended and transferred to a glass microscope slide in 13 mm circlular area.

Table – 3 DIFFERENCE BETWEEN THINPREP AND SUREPATH METHOD

	THINPREP	**SUREPATH**
Collecting Device	Brush is washed in the fixative and discarded	Bristle is detached into the fixative
Name of Fixative	PreserveCyt fluid	CytoRich fluid
Fixative Component	Methanol	Ethanol
Vortex	No vortex	Vortex mixed
Gradient Centrifuge	No gradient centrifugation	gradient centrifugation
Sedimentation	No sedimentation	Sedimentation
Filter	Filter used	No Filter used
Staining	Standard automated staining	Integral part of procedure
Smear Area	20mm	13mm

Figure – 2 Thin prep slide



Advantages of LBC [81,83]:

Advantages of LBC over conventional cervical cytology are

1. Simplifies the collection process for the smear taker.
2. Improves cellular preservation.
3. Reduces the rate of unsatisfactory cytology smears.
4. All the collected cells are transferred to the vial. Transfers 80-90% of cells to the slide as compared to 10- 20% with conventional pap smears.
5. Quicker for the laboratory to screen and report.
6. Distribute representative portion of cells on slide in uniform, even layer.
7. Residual cellular material could be used for ancillary studies.HPV assay.
8. Reduces the inflammatory cell background. Hence epithelial cell morphology can be better evaluated.

9. Multiple slides can be produced for teaching and quality Assurance purposes.
10. A possible reduction in specimen interpretation time.
11. LBC has at least as good sensitivity for detecting abnormalities.

Disadvantages:

The first generation LBC systems (ie:-ThinPrep and SurePath) requires

1. Filters, vaccums with automated equipment.
2. More expensive.
3. Smear taking procedures requires a standard approach.
4. Cytological interpretation differs from conventional methods and users have got to be trained

Second generation LBC:

This address the major issues of cost and instrumentation required in the first generation LBC**.**

Second generation LBC Liqui Prep has been introduced. Samples are collected using a cervical brush .The brush head is detached into the vial containing 5ml of preservative fluid . The sample is mixed with the vortex to form a homogenous mixture. Content in the fixative vial is poured in to the tube containing cleansing solution. and centrifuged at 1000 g for 10 min.

This cleaning solution separate the cells from the mucus and blood .The supernatant is discarded .To the cell base is added (4-5 volumes of cell button) and fully suspended by vortex mixing 50 µl of mixture is pippetted on to a slide in a circular motion and the slides are dried at room temperature and stained with Pap stain[84].

Advantages of LiquiPrep Method:

- Clean background
- Less expensive than first generation LBC method
- Ancillary studies like HPV can be done.

Liquid Based Cytology Smears:

Bethesda 2001 defined a new criteria for an adequate squamous component for both conventional and liquid based preparations.

Conventional smears : "Unsatisfactory" if <8000 well preserved and visualized squamous cells[85].

Liquid based preparations : adequate squamous component is 5000 well preserved and visualized cells [86]

- Minimum of 10 fields should be counted along a diameter that includes the center of the preparation. The average cell number per microscopic field to achieve 5000 cells.

For conventional cellular adequacy is based on cell pattern and not on the cell count. **Bethesda 2001-at least 10 well preserved endocervical and/or squamous metaplastic cells**[86].

Morphology of liquid based preparations [82]:

1. Clean background.
2. Cells are shrunken and are more circular.
3. Cytoplasmic and nuclear features are identified easily.
4. Cells with Low grade dyskaryosis , especially koilocytosis.
5. Nuclear hyperchromasia is lowered.
6. Tumor diathesis is found in a discrete pattern(clinging diathesis).
7. Glandular neoplasms show specific picture in thin film cytology.
8. In LBC endometrial cells appear slightly larger with more obvious nucleoli and enhanced chromatin pattern.
9. Atypical squamous cells of undetermined significance appear larger and flatter.
10. LSIL typically involves mature squamous cells with intermediate or superficial type. Cells of HSIL have more immature type cytoplasm .Overall cell size is smaller in HSIL as compared with LSIL.

11. The squamous cell carcinoma should be recognized due to its characteristic morphology and not due to tumour diathesis. LBC preparations are often characterized by lower tumour cellularity.

TABLE – 3 FEATURES OF KERATINIZING AND NON KERATINIZING SCC IN LAB PREPARATION

CORNIFIED TYPE(IN LBC)	**NONCORNIFIED TYPE(IN LBC)**
There is no difference in keratinized cells in comparison to conventional preparations	• Cytoplasm is more contracted &denser • The nucleus appears smaller • Chromatin is distributed more evenly • Nucleoli are more prominent • Malignant nuclear features are preserved

Follow up and management of abnormal cervical smears:

ASC – US

- Follow up by smears from the later
- HPV testing if negative – return to routine screening schedule (The screening interval should be extended to 3 years) and if positive – refer to Colposcopy.
- Postmenopausal women with atrophy may require oestrogen therapy before repeat smears a week after stoppage of regimen or may be referred for colposcopy or HPV testing without delay[84].

ASC-H:

- Colposcopic examination
- If no lesion is seen, refer the patient for follow up, HPV testing and cytology (every 6-12 months, possibly followed by diagnostic conization)[88].

LSIL

- Colposcopic examination
- HPV testing has been shown to be of no diagnostic or prognostic value before colposcopy.
- In postmenopausal women, estrogen therapy may be valve before colposcopy.
- In adolescents a conservative approach may be adopted

HSIL:

Colposcopic examination with biopsies[89]

If colposcopy is negative and on review, the cytological diagnosis is confirmed a diagnostic conization is indicated

Bjorn Strander et al [90] in their study by taking eight thousand eight hundred and ten conventional pap smears and 4674 liquid based samples. In the above study inadequate samples were observed in 0.3% of LBC and 0.7% of conventional pap smears. In the same study histolopathogic evaluation was made on 570 patients. Among them forty percent high grade lesions were identified in liquid based sampling , 1.2% in liquid based and 0.85% in conventional and it was concluded LBC yielded an increased rate of histolopathologic proven high grade lesions compared with conventional cytology.

J Rimiene et al [91] in their split sample study of 1,500 women and concluded the sensitivity of conventional was 68.7% and Papspin was 78.1% and specificity was 93.8% in conventional and 91.8% in Papspin and this method is a good alternative to conventional pap smear and offers several advantages for HPV- (DNA) testing and cell block preparations.

Guglielmo Ronco et al [92] in their study by screening 22466 in the conventional group and 22708 in the liquid based cytology they concluded that liquid based cytology detected more lesions of grade 1 and did not detect lesions of grade 3 or more and there was no statistically significant difference in sensitivity to conventional cytology for detection of cervical intraepithelial neoplasia of grade 2 or more , and a significant reduction in positive predictive value was found out.

Jung Dal Lee et al study[93] in comparing diagnostic cytomorphology of atypical squamous cells in liquid based preparations and conventional smears concludes that lower atypical squamous cells detection rates in conventional pap smears due to susceptibility to air –drying artifacts,that make cellular features difficult to interpret, whereas this problem is largely eliminated in liquid based preparations.

Anjali Limaye et al [94] in their study in comparing the thin prep preparation and conventional papanicolaou smears states that the false negative cases are significantly less in fluid based thin layer method.

Ovadia Abulafia et al [95] in their study concluded that Liquid based cytology is more sensitive in the detection of low grade and high grade squamous intraepithelial lesion .sensitivity rates were 68% in conventional

smears in relation to histology and76% in liquid based cytology, specificity rates were 79% in conventional smears and 86% in liquid based smears.

Azadeh Stark et al [96] in their study concluded that liquid based cytology yielded a significant reduction in unsatisfactory smears because of cellular inadequacy and excessive inflammation and the specimen rejected for evaluation is increased in liquid based smears.

Sara J Bernstein et al [97] in their prospective study he evaluated the cytological diagnosis and sample adequacy of the liquid based smears with conventional smears according to Bethesda system. Thin prep detect more low grade and high grade squamous intraepithelial lesions. There was no difference in the analysis of detecting atypical cells of undetermined significance. This Study proved Thin prep is superior to conventional pap smear.

Giovanni Negri et al [98] in their study of cytological follow up of cases atypical squamous cells,cannot exlude a high grade lesion(ASC-H) or atypical glandular cells(AGC)Thin prep reduce the occurance of ASC/AGC because of specimen adequacy. In his study of 214 cases ,Within normal limit, squamous intraepithelial lesion or carcinoma in58 cases, ASC/AGC in in 50 cases , and 6 smears were inadequate in conventional and WNL,SIL,

or carcinoma was diagnosed in 82 cases, ASC/AGC in 18 cases. No inadequate smears were found.

Jie zhu et al in their study [99] of 137 women with atypical pap smear in population based screening was done.The smears were compared with histological follow up. The detection rate of detecting high grade lesion was more 66%in thin prep than conventional.(47%). The correlation with histological diagnosis in Thin prep was53% and 37% in conventional. And it was concluded the detection of atypical squamous undetermined significance was less 4.5% in Thin prep and 8% in conventional.

Macharia H.C.et al[100] in their study of comparative analysis of conventional, liquid based cytology, colposcopy clinical impression with colposcopy biopsy histology concluded women referred with abnormal pap smear, requiring repeat pap smear liquid based cytology is used due to its higher specificity. Liquid based cytology showed better performance as a screening test for conventional pap smear

Annie N.Y. Cheung et al [101] in their study , with Thin prep unsatisfactory rate was reduced from 0.48% to 0.32%.There was an increase in the detection rate of atypical squamous cells of undetermined significance and low grade squamous intra epithelial lesion. And decrease in the ASCUS

to LSIL ratio from3.15 for conventional and 2.33 for thin prep. In Thin prep more cases of actinomyces were detected.

Beerman et al [102], in their study, liquid based cytology and conventional cytology were done in two groups and concluded sensitivity for detection of a histologically proven lesion is higher in liquid cohort compared to conventional cohort 96.2% aVs92.0%

Sherwani RK et al [103] in his study of 160 cases infectious agents were detected in 8.7% cases in liquid based cytology and 3.1% in conventional pap smear. No cytological abnormality was found in women who started their sexual activity after 25 years of age

Luis A et al[104] in their study the detection rate of low grade squamous intraepithelial lesions increased by71.65% and detection of high grade squamous intraepithelial lesions increased by 102.54% and decrease in the atypical squamous cells of undetermined significance from 2.07 to 1.26.

J Monsonego et al [105] in their study concluded 29% more ASCUS cases and 39% more low grade squamous intraepithelial lesions were detected in thin prep than conventional smears.

PE castle et al [106] in their study of detecting the rate of unsatisfactory smears in liquid based cervical samples and conventional, the reduction of unsatisfactory slides with LBC was greatest in younger women and decreased with increasing age compared with conventional smears.

Mojgan Karimi-Zarchi et al [107] in their study of 150 patients, of Atypical Squamous Cells in previous pap smearpatients, conventional pap smear, liquid based cytology smear, colposcopy and cervical biopsy were done. Conventional pap smear method had a sensitivity 51%,specificity 66.6%,PPV 96%,NPV 8% and accuracy was 92%,liquid based cytology smear method sensitivity was51%,specifitywas 77.7%,PPV was 97.5%,NPV was 10% and accuracy was 56.6% .In the coloscopy sensitivity was 70/9%, specificity was 44/4%, PPV was 95.2%,NPV was 8/8% and accuracy was 69.3%.compared with other diagnostic techniques colposcopy has the best efficacy in detecting cervical lesions.

Hopwood et al [108] in their study concluded that Thin prep processor provide a platform for Chlamydia screening and there was no cross contamination.

Gulielmo Ronco et al [109] in their study, combined use of HPV and liquid based cytology, led to a 60% loss in PPV. Increase in sensitivity were still obtained with HPV alone at 1 and 2 pg/mL cutoffs (42% and 41%,

respectively, compared with conventional cytology), and these approaches reduced the losses in PPV (42& and 25% respectively, compared with conventional cytology). Liquid-based cytology did not cause an increase in sensitivity compared with conventional cytology but led to a substantial (43%) reduction in PPV.

Methodology

METHODOLOGY

The present study was conducted in the department of Pathology, Sree Mookambika Institute of Medical sciences, Kulasekharam, Kanyakumari District, Tamilnadu and the study samples were taken from the department of Obstetrics and Gynecology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari District.

Study design:

This study is a cross sectional study on cervical exfoliative cytology smears.

Study setting:

The study were selected from the eligible population came to the out patient department of Obstetrics and Gynecology, during the study period.

Duration of study:

This study was done during the time period between April 2013 to March 2014

Sample size calculation:

Sample size was calculated using EZR on R commander (3.0.0)

Calculate sample size for non inferiority trial of two proportions :

	Assumptions.
P1	0.90
P2	0.95
Delta	0.05
Alpha	0.05
	two sided
Power	0.8
Estimated	
Required sample size	109

Sample size :

110 samples were selected for the study for them both conventional and liquid based cytology smears were done.

Inclusion Criteria:

1. Women in the age group of 25 – 60 years.
2. Women attending to gynecology out patient department with any of the below conditions:

- Abnormal vaginal discharge
- Post coital bleeding
- Persistant leucorrhea
- Abnormal uterine bleeding
- Postmenopausal bleeding
- Any other abnormal findings on speculum examination.

Exclusion criteria:

The following catagories

1. Those who are not willing to participate
2. Unmarried women
3. Pregnant women
4. Post- hysterectomy
5. Post -chemotherapy / radiotherapy

Parameters:

The pathological features of the slides for both the methods were observed.

The Background:

Adequacy of the specimen:

- Satisfactory for evaluation
- Unsatisfactory

General Categorization:

- Within normal limits
- Epithelial cell abnormality

Benign cellular changes:

- Infection
- Reactive atypia

Epithelial cell abnormality:

- Atypical squamous cell of undetermined significance (ASCUS)
- Atypical squamous cell -cannot exclude high grade lesion(ASC-H)

Glandular cell abnormalities (NOS):

- Low grade squmous intraepithelial lesion (LSIL)
- High grade squamous intraepithelial lesion(HSIL)
- Invasive Cancer, Squamous cell carcinoma, others.
- The relavant clinical data were collected.

Ethical consideration:

The study proposal was submitted to Institutional Human Ethics Committee [IHEC] of Sree Mookambika Institute of Medical Sciences [SMIMS] Kulasekharam [Kanyakumari District], Tamilnadu for approval and the research proposal was approved by the Institutional Human Ethics

Committee [IHEC] of SMIMS with **Ref. No. SMIMS/IHEC/2013/A/06.** The certificate of approval for the same has been enclosed in annexure.

The Gynecologist and cytopathologist were a part of the study team who worked together towards one common aim that is improving patient compliance and hence ensuring proper and early treatment there by helping the patient. For this purpose efforts were made to take adequate smears and to reduce the number of unsatisfactory smears. This led to the invention of newer technologies liquid based cytology that caused a reduction in the number of unsatisfactory smears.

Procedure:

Written and informed consent were obtained from all participants after brief explanation of the procedure.

General instructions:

Before obtaining an ideal pap smear specimen, patients are instructed to avoid vaginal medications, vaginal contraceptives or douches for 48 hours before the appointment. Sexual intercourse is not recommended, the night before the appointment. Smears are taken 2 weeks after the first day of the last menstrual period.

Preparing to take sample:

- The aim of taking sample is explained to women
- Detailed history about the symptoms
- Obstetric history and menstrual history obtained
- For conventional label the slide with patient name and number
- For LBC, label the vial with same information
- Position of the patient is made comfortable.

The sample should be obtained before the application of acetic acid or lugols iodine. The patient was placed in dorsal position, the labia separated .After visualizing the cervix, the Cusco's self retaining speculum was gently inserted without the use of lubricant or jelly. The cervix was exposed and visualized for any gross pathological features under adequate light and findings were recorded.

Pap smear:

After preliminary inspection of the cervix, excess mucus or other discharge should be removed. The Ayres spatula is inserted into the cervical canal, the squamo columnar junction was scrapped with the Ayres spatula and rotated 360 degree. The material is immediately spread on to labeled glass slides and immediately fixed with 80% isopropyl alcohol and sent to

the cytopathological laboratory. The smears are then stained by Papanicolaou method.(Annexure).

Simultaneously for liquid based cytology cerviex brush is used to collect the sample. Insertion of long bristles into the endocervical canal, and short bristles against ectocervix and rotated 360°. Each brush is rinsed in a vial containing Preserv Cyt solution. The Thin Prep sample vial is then capped, labeled and sent to laboratory.

Thin Prep Method:

In the laboratory the sample is then matched to the corresponding cytology requisition form .The vial and the matching slide are placed into the Thin Prep Processor along with a disposable Gynecological filter. The instrument homogenizes the sample by spinning the filter. Shear forces created during spinning break up blood, mucus and debris keeping the true cell clusters intact. Then the liquid preservative solution is filtered through a membrane filter with a pore size specifically designed to trap epithelial cells while allowing contaminating red blood cells and inflammatory cells to pass through. The epithelial cells collected on the membrane filter are then transferred onto a glass slide in circle of 20mm diameter. After that the slides are dried and stained by automated stainer. This produces a relatively thin, monolayer-type preparation.

The 2001 – Bethesda system was used for reporting of cervical cytology smears.(Annexure)

Figure – 4 THIN PREP 2000 PROCESSOR

Statistical analysis:

The data collected were entered into the Microsoft Office Excel 2007 for Windows7. Statically analysis was done using SPSS. Version 16.Simple percentages, proportions, mean, stranded deviation were calculated. Test of significant like chi-square test was used two identify the difference pattern between two smears. p value < 0.05 was taken as significant. The results were presented in tables and appropriate diagrams.

Results

5.RESULTS

5.1 GENERAL CHARACTERISTICS OF THE STUDY POPULATION

Table - 5.1.1 Age

Age Category	Frequency	Percent
1. Up to 30 Years	4	3.6
2. 31 to 40 Years	28	25.5
3. 41 to 50 Years	65	59.1
4. 51 to 60 Years	13	11.8
Total	110	100

In our study of 110 women, who have been screened majority in the age group 41-50 years were 65 (59.1%), women between the age group of 31-40 years were 28 (25.5%), women between the age group of 51-60 years were 13 (11.8%). Those in the age group up to 30 are 4 (3.6%).

Fig 5.1.1 Showing the Age Category of the study population

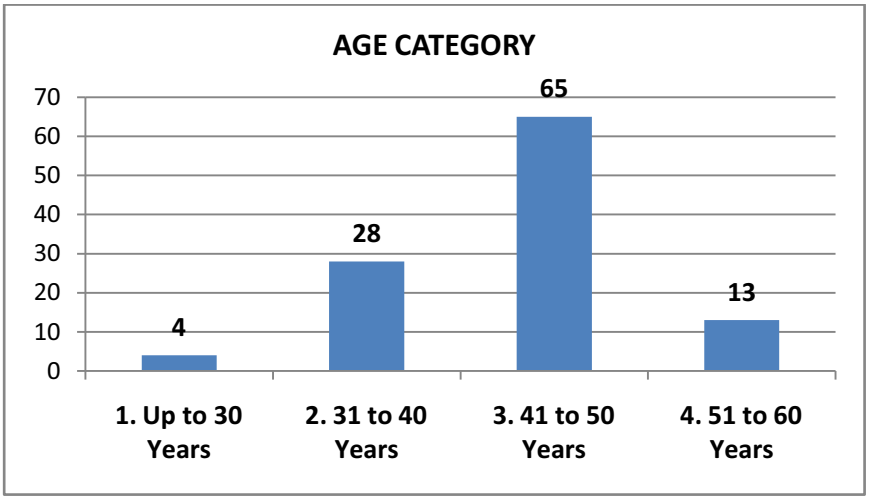


Table - 5.1.2 Education

	Frequency	Percent
1. Primary	10	9.1
2. Middle school	14	12.7
3. High school	4	3.6
4. HSS	73	66.4
5. Degree	9	8.2
Total	110	100

In our study most of the women had education up to higher secondary school were 73 (66.4 %). Women had education up to middle school are 14 (12.7%). 10 (9.1%) had education up to primary, 9(8.2%) had education up to degree and the rest 4 (3.6%) had education up to high school.

Fig 5.1.2 Showing the Education Category of the study population

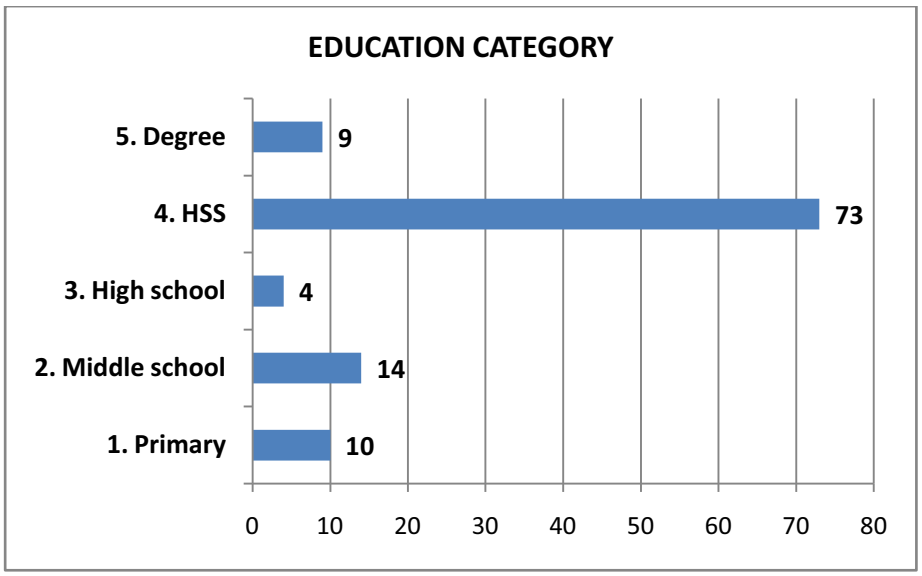


Table - 5.1.3 Occupation

	Frequency	Percent
1. Housewife	76	69.1
2. Cooley	16	14.5
3. Employed	18	16.4
Total	110	100

In our study most of the women were housewives 76 (69.1%). 18 (16.4%) were employed and women of 16 (14.5%) were cooley.

Fig 5.1.3 Showing Occupation of the study population

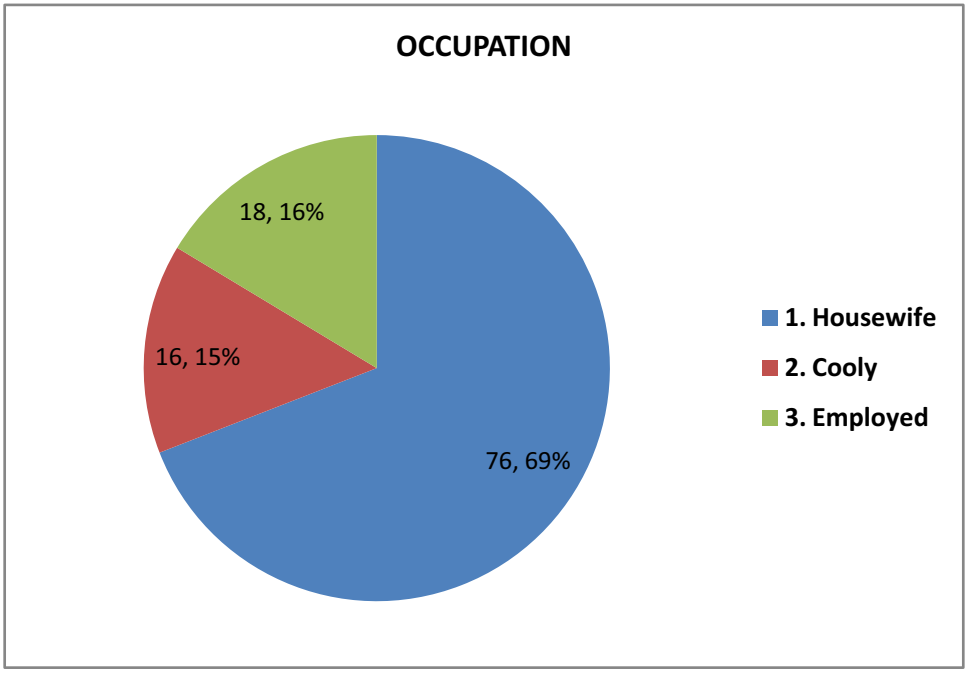


Table - 5.1.4 Number of Deliveries

	Frequency	Percent
1.Nil	6	5.5
2.One	17	15.5
3.Two	57	51.8
4.Three	22	20.0
5. Four	7	6.4
6. Five	1	0.9
Total	110	100

In our study of 110 women, majority of the women who had two deliveries were 57(51.8%). Women who had three deliveries were 22 (20%). Women with one delivery were 17(15.5%). 7(6.4%) had four deliveries. 1 (0.9%) had five deliveries and women without any delivery were 6 (5.5%).

Fig 5.1.4 Showing the Number of Deliveries

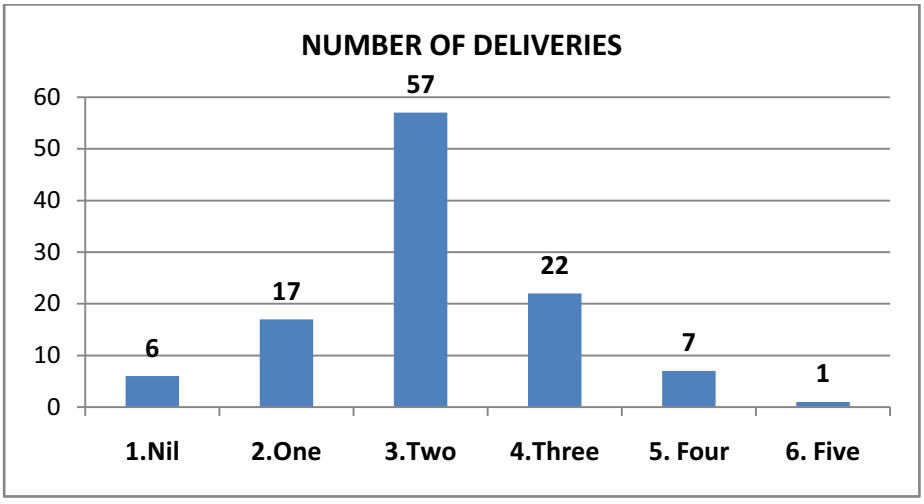


Table - 5.1.5 Gap between Deliveries

	Frequency	Percent
1 Year	20	23.0
2 Year	60	69.0
3 Year	5	5.7
4 Year	2	2.3
Total	87	100

Among the 110 women, majority of the women had a gap of 2 years between their deliveries were 69.0%. Women who had a gap of 1 year between their child births were 23%, women who had a gap of 4 years were 2.3% and women who had a gap of 3 years were 5.7%

Fig 5.1.5 Showing Gap between Deliveries

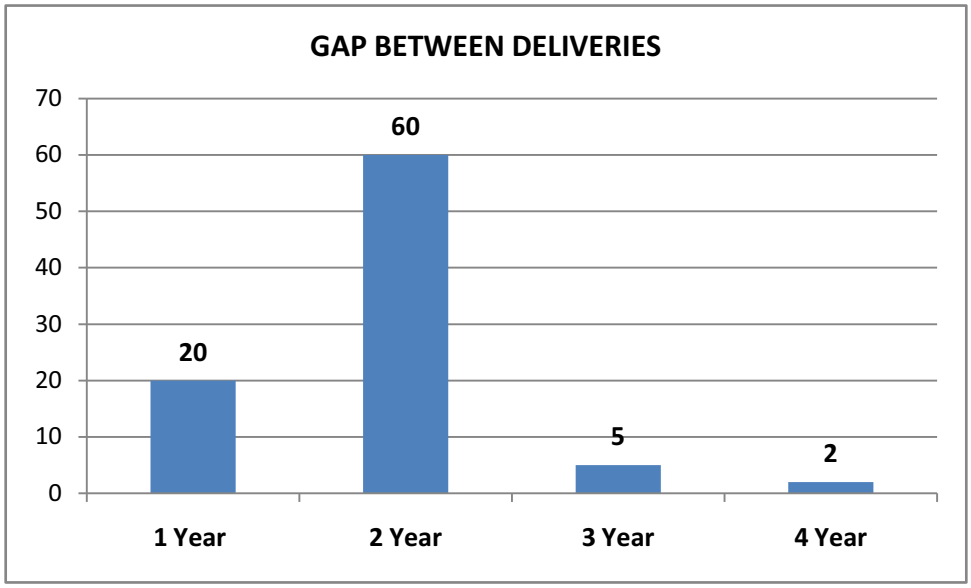


Table - 5.1.6 History of Cu-T Insertion

	Frequency	Percent
1.Yes	7	6.4
2. No	103	93.6
Total	110	100

In our study, 7(6.4%) women had a history of Copper-T Use and 103 (93.6%) women had no history of Copper – T use.

Fig 5.1.6 Showing History of Cu-T Insertion

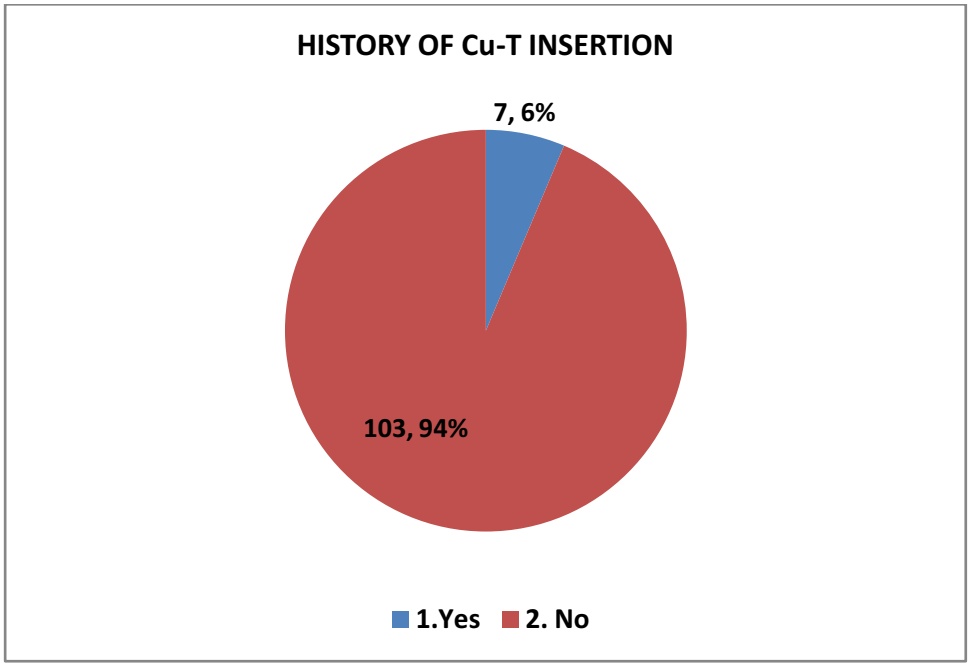


Table - 5.1.7 History of STD / HIV in Patient

	Frequency	Percent
1.Yes	0	0.0
2. No	110	100
Total	110	100

In our study no women had history of HIV.

Table - 5.1.8 History of STD / HIV in Partner

	Frequency	Percent
1.Yes	0	0.0
2. No	110	100
Total	110	100

In our study no women had partners with history of HIV.

Table 5.1.9 History of Chronic Vaginal Infection

	Frequency	Percent
1.Yes	13	11.8
2.No	97	88.2
Total	110	100

In our study, 13(11.8%) had history of chronic vaginal infection and 97(88.2%) without history of infection.

Fig 5.1.9 Showing History of Chronic Vaginal Infections

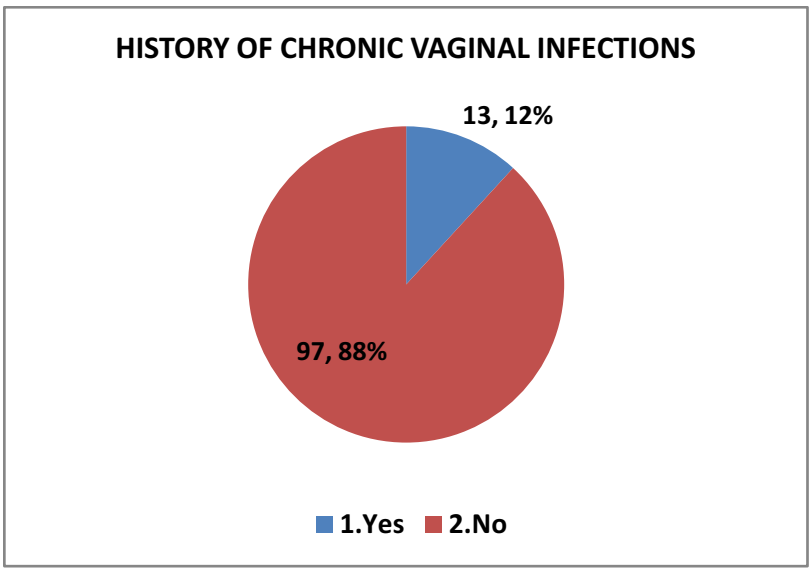
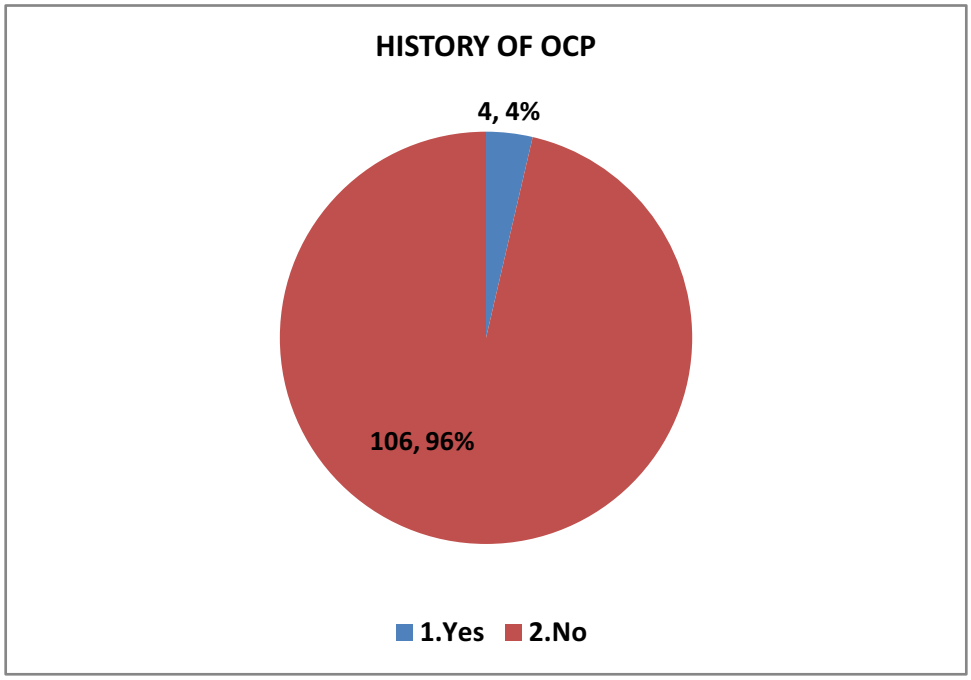


Table - 5.1.10 History of OCP Usage

	Frequency	Percent
1.Yes	4	3.6
2.No	106	96.4
Total	110	100

In our study, women with history of OCP usage were 4(3.6%) and without usage were 106 (96.4%).

Fig 5.1.10 Showing History of OCP



5.2 DIAGNOSIS OF LESIONS

Table - 5.2.1 Lesions by Conventional Method

	Frequency	Percent
1.Unsatisfactory	6	5.5
2. Normal	8	7.3
3. Inflammatory	89	80.9
4. Atrophic	3	2.7
5. ASCUS	1	0.9
6. LSIL	2	1.8
7. HSIL	1	0.9
Total	110	100

In our study, among the 110 conventional pap smears, unsatisfactory smears were 6 (5.5%), normal smears were 8 (7.3%), smears characterized as inflammatory were 89 (80.9%), and atrophic smears were 3 (2.7%), smears with features of ASCUS were 1(0.9%), smears with features of LSIL were 2 smears (1.8%),smears with features of HSIL were1 (0.9%)

Fig - 5.2.1 Lesions by Conventional Method

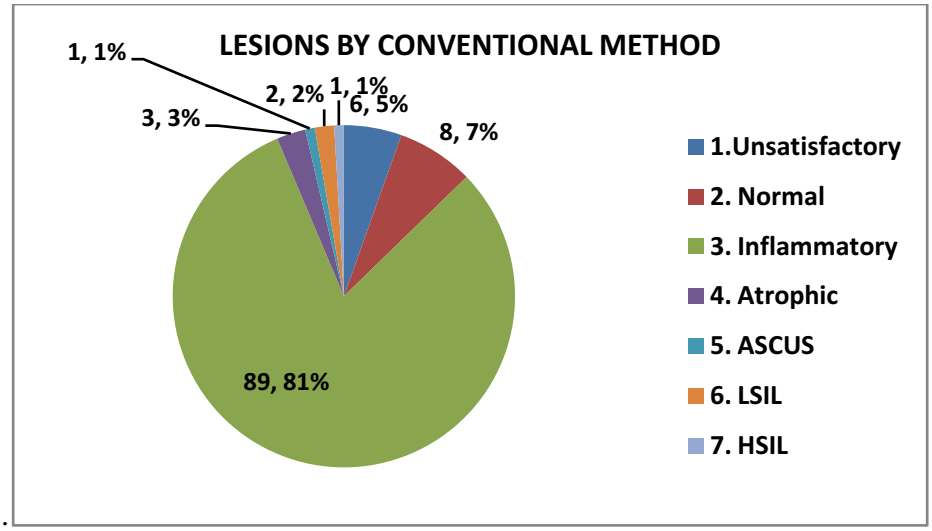


Table - 5.2.2 Lesions by Liquid Based Cytology

	Frequency	Percent
1.Unsatisfactory	0	0.0
2. Normal	9	8.2
3. Inflammatory	88	80.0
4. Atrophic	3	2.7
5. ASCUS	5	4.5
6. LSIL	4	3.6
7. HSIL	1	0.9
Total	110	100

In our study among the LBC smears, there were no unsatisfactory smears (0%),normal smears were 9(8.2%),smears characterized as inflammatory were 88(80.0%),atrophic smears were3(2.7%),smears with features of ASCUS were 5(4.5%),smears with features of LSIL were4(3.6%), and 1(0.9%) smear with features of HSIL were 1(0.9%)

Fig - 5.2.2 Lesions by Liquid Based Cytology

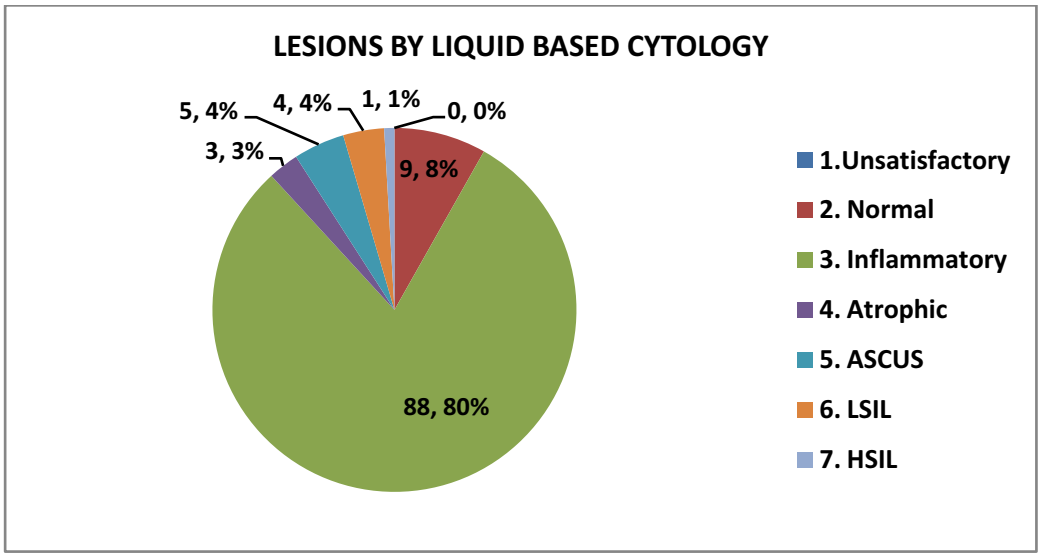


Table - 5.2.3 Comparison of Lesions by Liquid Based Cytology with Conventional Method

LESIONS	CONVENTIONAL METHOD	LIQUID BASED CYTOLOGY	Chi^2 Value	p-Value
1.Unsatisfactory	6	0	4283	0.0385
2. Normal	8	9	0.064	1.801
3. Inflammatory	89	88	0.029	0.865
4. Atrophic	3	3	0.000	1.000
5. ASCUS	1	5	1.542	0.214
6. LSIL	2	4	0.171	0.679
7. HSIL	1	1	0.000	1.000

Chi^2 Value – 4283 df - 5 p-Value – 0.0385

Tables show comparision of lesions by conventional and liquid based cytology. According to Chi square value, p value of each category, unsatisfactory lesions are significantly higher in conventional methods and none other categories are significant. p value obtained is0.03,it is statistically significant.

Fig 5.2.3 Comparison of Lesions by Liquid Based Cytology with Conventional Method

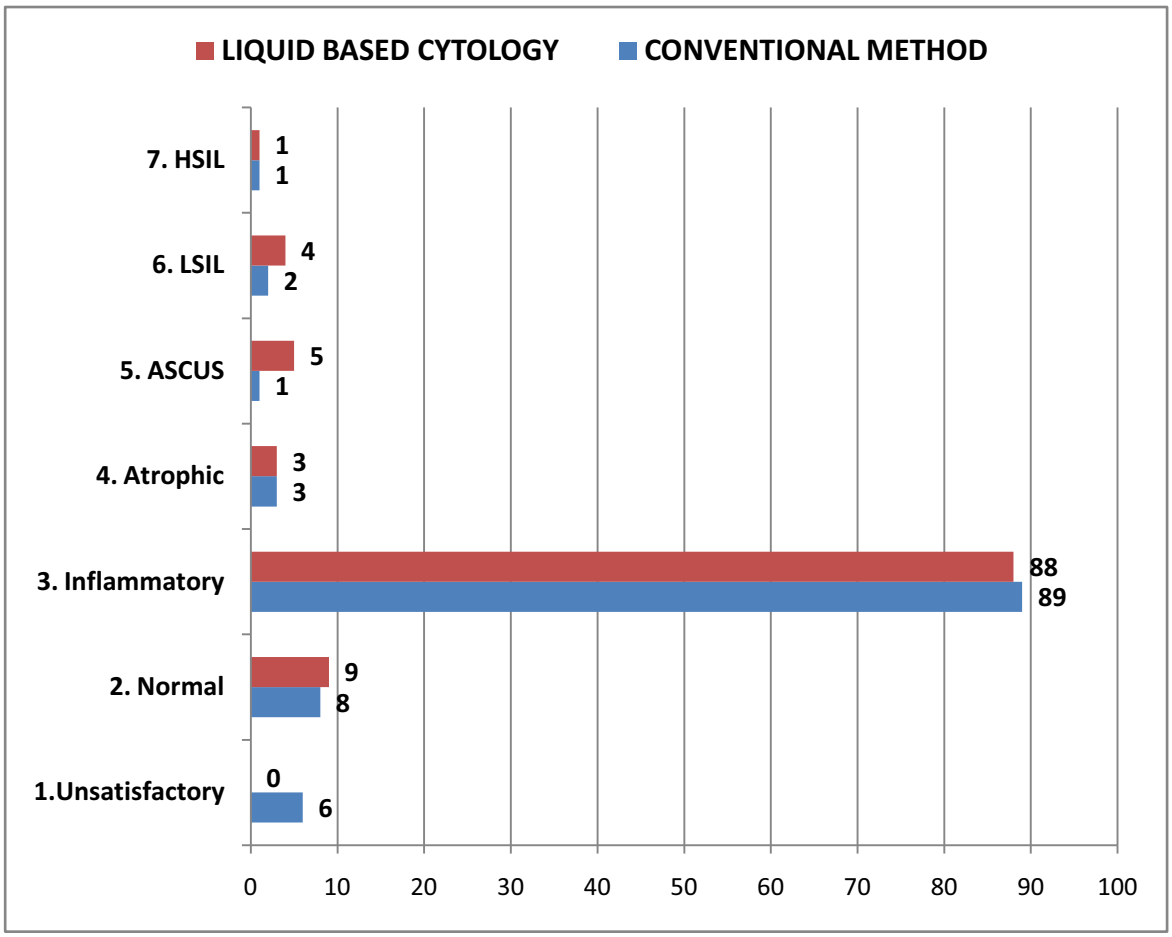


Table shows the comparison of Liquid Based Cytology smears with conventional smears. The unsatisfactory smears were found to be more in conventional and abnormal smears were found to be more in liquid based cytology .

5.3 DISTRIBUTION OF LESIONS IDENTIFIED BY LIQUID BASED CYTOLOGY

Table - 5.3.1 Distribution of Lesions by Age Category

LESIONS	AGE CATEGORY		TOTAL
	LOWER	HIGHER	
Normal	3 (9.4%)	6 (7.7%)	9 (8.2%)
Inflammatory	25 (78.1%)	63 (80.8%)	88 (80.0%)
Atrophic	2 (6.2%)	1 (1.3%)	3 (2.7%)
ASCUS	1 (3.1%)	4 (5.1%)	5 (4.5%)
LSIL	1 (3.1%)	3 (3.8%)	4 (3.6%)
HSIL	0 (0.0%)	1 (1.3%)	1 (0.9%)
Total	32 (100%)	78 (100%)	110 (100%)

Chi^2 Value – 1.939 df - 5 p-Value – 0.858

Lesions detected in the lower age group, normal smears were 3 (9.4%), inflammatory were 25 (78.1%), atrophic smears were 2(6.2%), smears detected as ASCUS were 1(3.1%), smears detected as LSIL were1(3.1%), HSIL were 0(0.0%). Lesions detected in the higher age group, the normal smears were 6(7.7%), inflammatory were 63 (80.8%), atrophic were 1(1.3%), smears detected as ASCUS were 4(5.1%), LSIL were 3

(3.8%), HSIL were 1 (1.3%). p value obtained is 0.8, it is not statistically significant.

Table - 5.3.3 Distribution of Lesions by Age at Marriage Category

LESIONS	AGE AT MARRIGAE		TOTAL
	LOWER	HIGHER	
Normal	3 (7.3%)	6 (8.7%)	9 (8.2%)
Inflammatory	32 (78.0%)	56 (81.2%)	88 (80.0%)
Atrophic	1 (2.4%)	2 (2.9%)	3 (2.7%)
ASCUS	2 (4.9%)	3 (4.3%)	5 (4.5%)
LSIL	2 (4.9%)	2 (2.9%)	4 (3.6%)
HSIL	1 (2.4%)	0 (0.0%)	1 (0.9%)
Total	41 (100%)	69 (100%)	110 (100%)

Chi^2 Value – 2.087 df - 5 p-Value – 0.837

Lesions detected by marriage age in the lower age group, the normal smears were 3 (7.3%), inflammatory were 32 (78.0%), atrophic smears were 1(2.4%), smears detected as ASCUS were 2 (4.9%), LSIL were 2(4.9%), HSIL were 1(2.4%). Lesions detected in the higher age group, normal smears were 6(8.7%), inflammatory were 56 (81.2%), atrophic smears were 2 (2.9%), ASCUS were 3 (4.3%), LSIL were 2 (2.9%), HSIL were 0 (0.0%). Since the P value obtained is 0.8, it is not statistically significant.

Table - 5.3.4 Distribution of Lesions by Number of Deliveries Category

LESIONS	NUMBER OF DELIVERIES		TOTAL
	LOWER	HIGHER	
Normal	6 (7.5%)	3 (10.0%)	9 (8.2%)
Inflammatory	63 (78.8%)	25 (83.3%)	88 (80.0%)
Atrophic	3 (3.8%)	0 (0.0%)	3 (2.7%)
ASCUS	3 (3.8%)	2 (6.7%)	5 (4.5%)
LSIL	4 (5.0%)	0 (0.0%)	4 (3.6%)
HSIL	1 (1.2%)	0 (0.0%)	1 (0.9%)
Total	80 (100%)	30 (100%)	110 (100%)

Chi^2 Value – 3.632 df - 5 p-Value – 0.603

Lesions detected by the number of deliveries in the lower age group, normal smears were 6(7.5%),inflammatory smears were 63(78.8%),atrophic smears were 3(3.8%),smears detected as ASCUS were 3(3.8%),LSIL were 4(5.0%),HSIL were 1 (1.2%) and in the higher age group ,normal smears were 3(10.0%),inflammatory smears were25 (83.3%),atrophic smears were 0(0.0%),ASCUS were 2(.7%),LSIL were 0(0.0%),HSIL smears were 0(0.0%). p value obtained is 0.6, it is not statistically significant.

Table - 5.3.5 Distribution of Lesions by Gap at Deliveries Category

LESIONS	GAP AT DELIVERIES CATEGORY			TOTAL
	LOWER	HIGHER	NOT APPLICABLE	
Normal	1 (5.0%)	7 (10.4%)	1 (4.3%)	9 (8.2%)
Inflammatory	17 (85.0%)	50 (74.6%)	21 (91.3%)	88 (80.0%)
Atrophic	1 (5.0%)	2 (3.0%)	0 (0.0%)	3 (2.7%)
ASCUS	1 (5.0%)	4 (6.0%)	0 (0.0%)	5 (4.5%)
LSIL	0 (0.0%)	3 (4.5%)	1 (4.3%)	4 (3.6%)
HSIL	0 (0.0%)	1 (1.5%)	0 (0.0%)	1 (0.9%)
Total	20 (100%)	67 (100%)	23 (100%)	110 (100%)

Chi^2 Value – 5.660 df - 10 p-Value – 0.843

Lesions detected by the gap between the deliveries, in the lower age group ,normal smears were 1 (5.0%),inflammatory were17 (85.0%),atrophic smears were 1 (5.0%),smears detected as ASCUS were 1 (5.0%),LSIL were 0 (0.0%),HSIL were 0 (0.0%).In the higher age group, normal smears were 7 (10.4%),Inflammatory were 50 (74.6%),atrophic were 2 (3.0%),ASCUS were 4 (6.0%),LSIL were 3 (4.5%) ,HSIL were 1 (1.5%). p value obtained is 0.8, it is not statistically significant.

Table - 5.3.5 Distribution of Lesions by Cu-T Insertion Category

LESIONS	Cu-T INSERTION CATEGORY		TOTAL
	YES	NO	
Normal	0 (0.0%)	9 (8.7%)	9 (8.2%)
Inflammatory	6 (85.7%)	82 (79.6%)	88 (80.0%)
Atrophic	0 (0.0%)	3 (2.9%)	3 (2.7%)
ASCUS	1 (14.3%)	4 (3.9%)	5 (4.5%)
LSIL	0 (0.0%)	4 (3.9%)	4 (3.6%)
HSIL	0 (0.0%)	1 (1.0%)	1 (0.9%)
Total	7 (100%)	103 (100%)	110 (100%)

Chi^2 Value – 2.746 df - 5 p-Value – 0.739

Lesions with history of cu –T insertion, normal smears were 0 (0.0%), inflammatory were 6 (85.7%), atrophic were 0 (0.0%), ASCUS were 1 (14.3%), LSIL were 0 (0.0%), HSIL were 0 (0.0%). Lesions with no history of copper-T insertion, normal smears were 9(8.7%), inflammatory were 82(79.6%), atrophic were 3 (2.9%), ASCUS were 4 (3.9%), LSIL were 4 (3.9%), HSIL were 1 (1.0%). p value obtained is 0.7, it is not statistically significant.

Table - 5.3.5 Distribution of Lesions by Chronic Vaginal Infection Category

LESIONS	CHRONIC VAGINAL INFECTON		TOTAL
	LOWER	HIGHER	
Normal	4 (30.8%)	5 (5.2%)	9 (8.2%)
Inflammatory	8 (61.5%)	80 (82.5%)	88 (80.0%)
Atrophic	0 (0.0%)	3 (3.1%)	3 (2.7%)
ASCUS	1 (7.7%)	4 (4.1%)	5 (4.5%)
LSIL	0 (0.0%)	4 (4.1%)	4 (3.6%)
HSIL	0 (0.0%)	1 (1.0%)	1 (0.9%)
Total	13 (100%)	97 (100%)	110 (100%)

Chi^2 Value – 11.214 df - 5 p-Value – **0.047** (Significant)

Lesions with history of chronic vaginal infection in lower age group, normal smears were 4(30.8%), inflammatory were 8(61.5%), atrophic were 0(0.0%), ASCUS were 1(7.7%), LSIL were 0(0.0%),HSIL were 0(0.0%). Lesions in the higher age group, normal were 5(5.2%), inflammatory were 80(82.5%), atrophic were 3(3.1%),ASCUS were 4(4.1%), LSIL were 4(4.1%), HSIL were 1(1.0%). p value obtained is 0.047. It is statistically significant.

Table - 5.3.5 Distribution of Lesions by History of OCP Category

LESIONS	HISTORY OF OCP		TOTAL
	YES	NO	
Normal	1 (25.0%)	8 (7.5%)	9 (8.2%)
Inflammatory	3 (75.0%)	85 (80.2%)	88 (80.0%)
Atrophic	0 (0.0%)	3 (2.8%)	3 (2.7%)
ASCUS	0 (0.0%)	5 (4.7%)	5 (4.5%)
LSIL	0 (0.0%)	4 (3.8%)	4 (3.6%)
HSIL	0 (0.0%)	1 (0.9%)	1 (0.9%)
Total	4 (100%)	106 (100%)	110 (100%)

Chi^2 Value – 1.939 df - 5 p-Value – 0.858

Lesions with history of usage of oral contraceptive pills, normal were 1(25.0%), inflammatory were 3(75.0%), atrophic, ASCUS, LSIL, HSIL were o(0.0%). Lesions without history of OCP , normal were 8(7.5%), inflammatory were 85(80.2%), atrophic were 3(2.8%), ASCUS were 5(4.7%), LSIL were 4(3.8%), HSIL were 1(0.9%). p value obtained is 0.8, it is not statistically significant.

Cytology Pictures

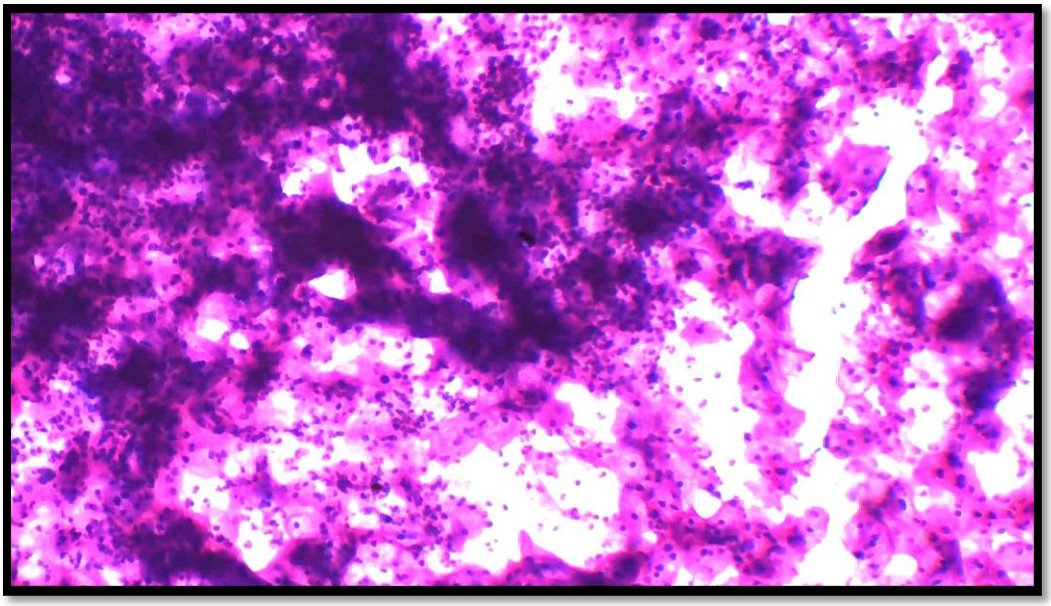


FIG- 4 CONVENTIONAL PAP - THICK SMEAR (40X)

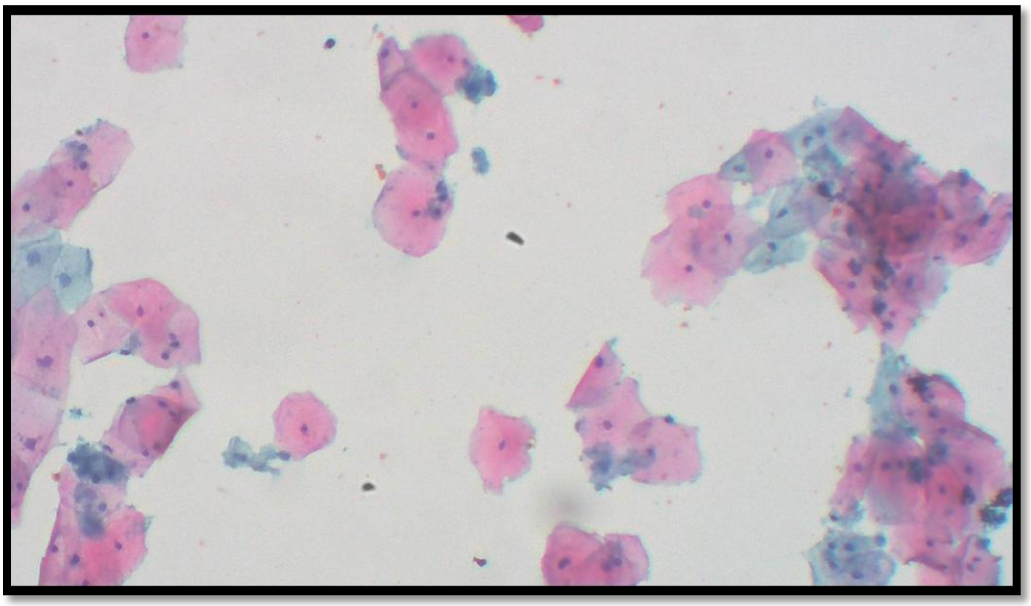


FIG- 5 LBP- NORMAL SMEAR (40X)

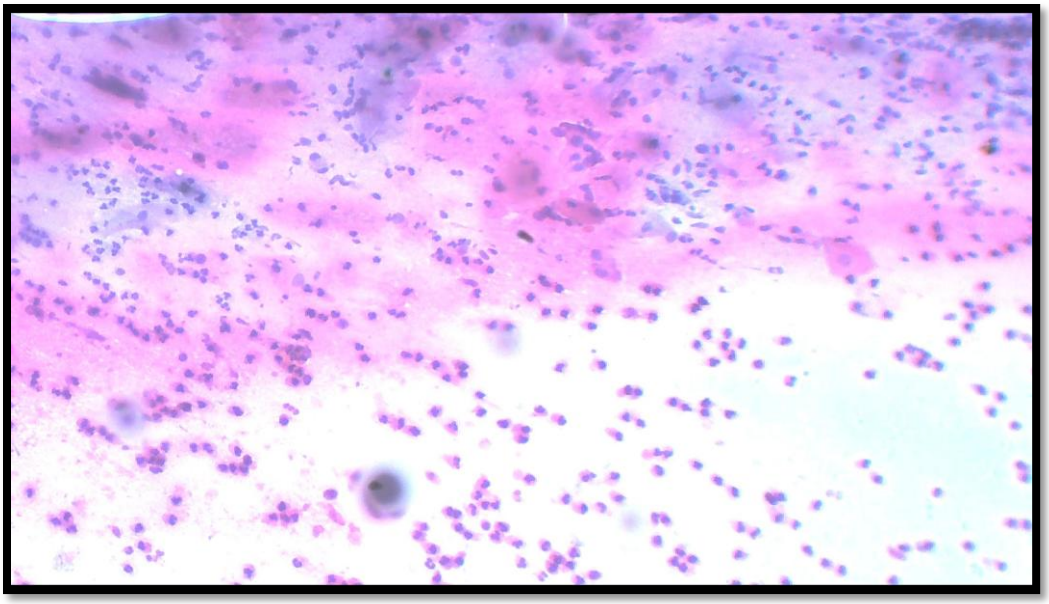


FIG – 6 CONVENTIONAL PAP - INFLAMMATORY SMEAR (10X)

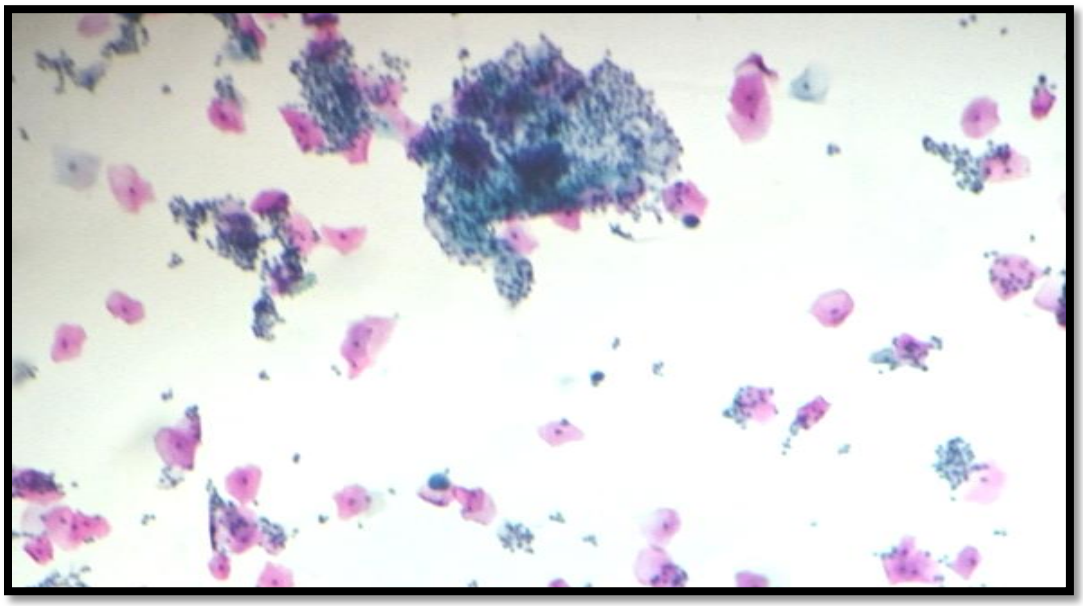


FIG – 7 LBP –INFLAMMATORY SMEAR (10X)

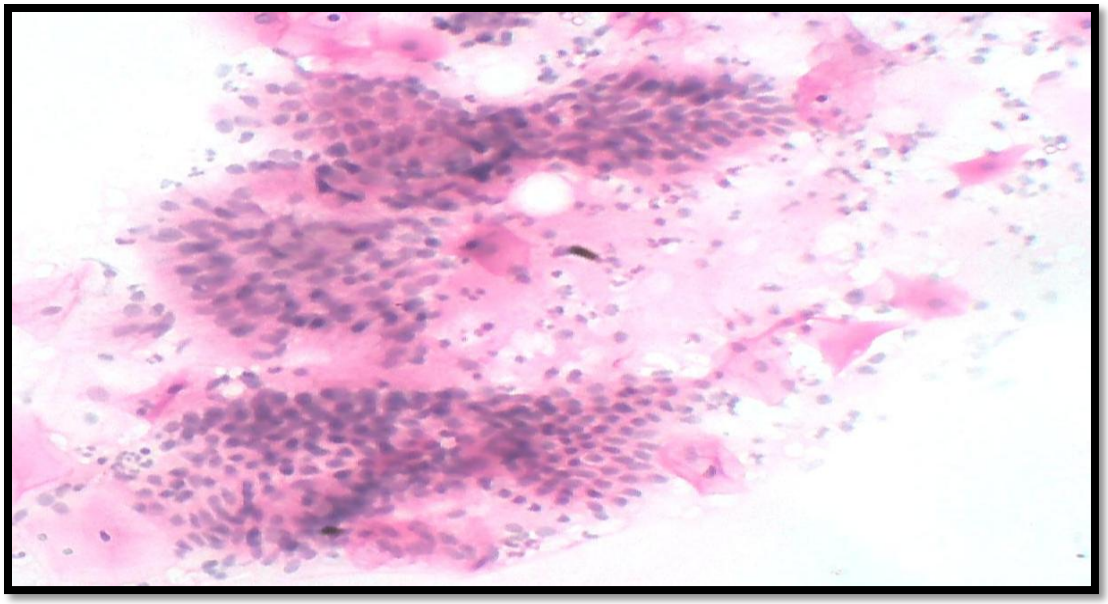


FIG – 8 CONVENTIONAL PAP - ENDOCERVICAL CELL CLUSTER (40 X)

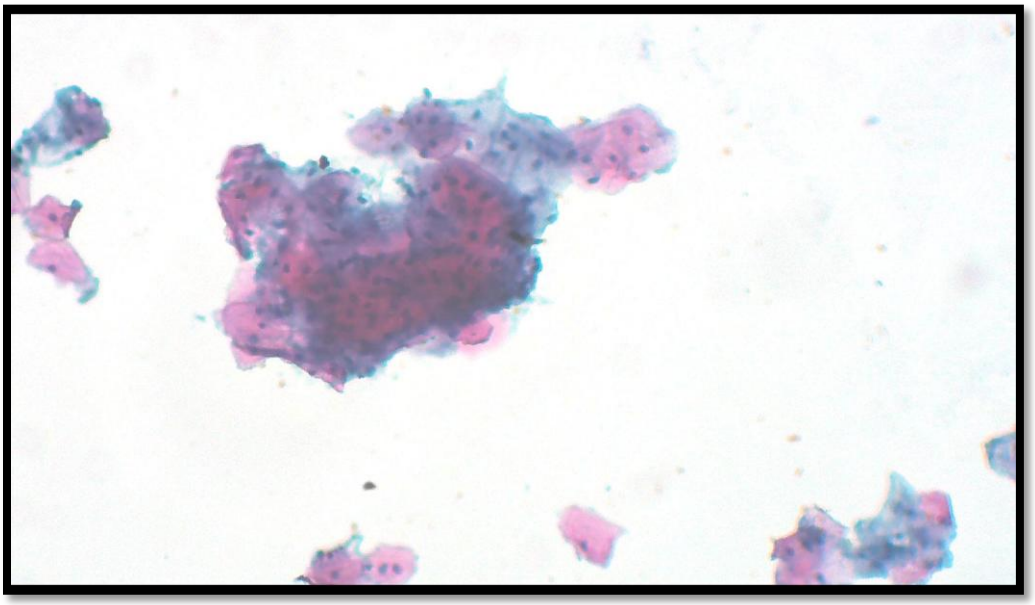


FIG – 9 LBP - ENDOCERVICAL CELL CLUSTER (40X)

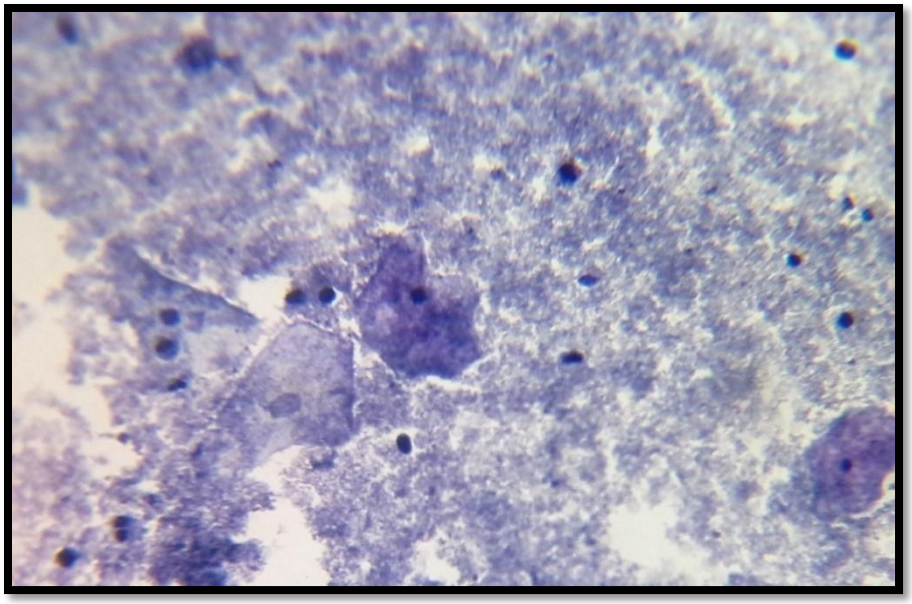


FIG – 10 CONVENTIONAL PAP –BACTERIAL VAGINOSIS (40X)

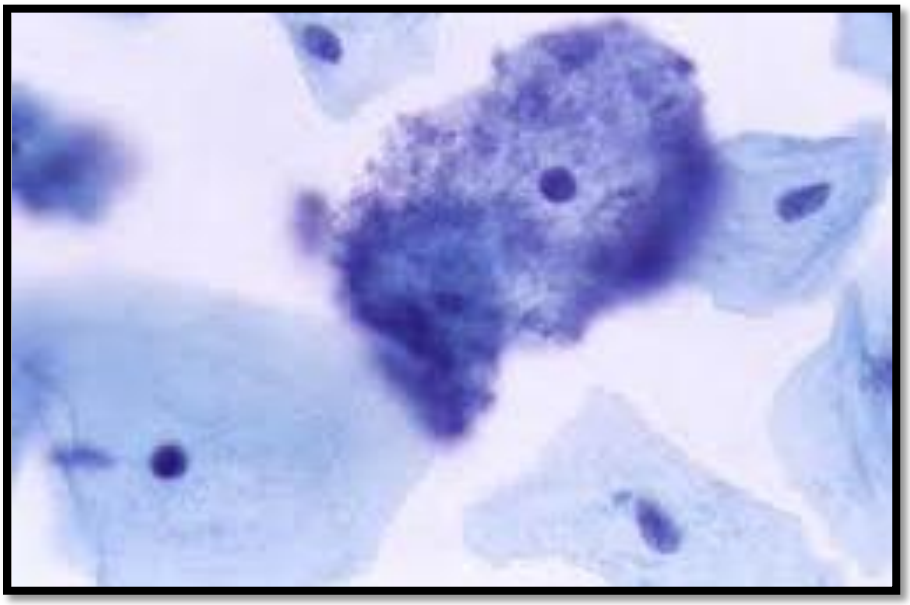


FIG – 11 LBP -BACTERIAL VAGINOSIS (High Power View)

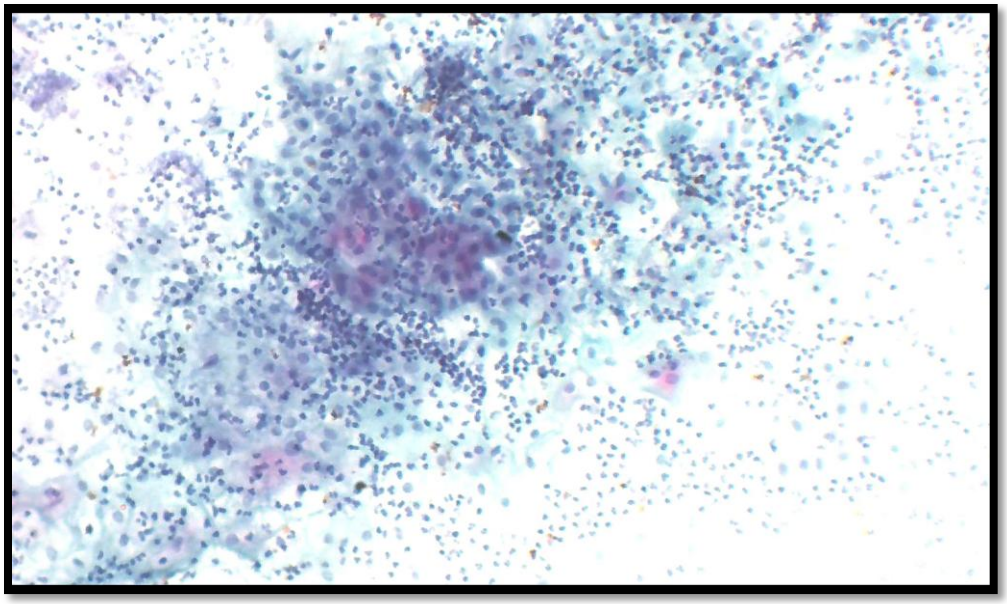


FIG – 12 CONVENTIONAL PAP –ASCUS (40X)

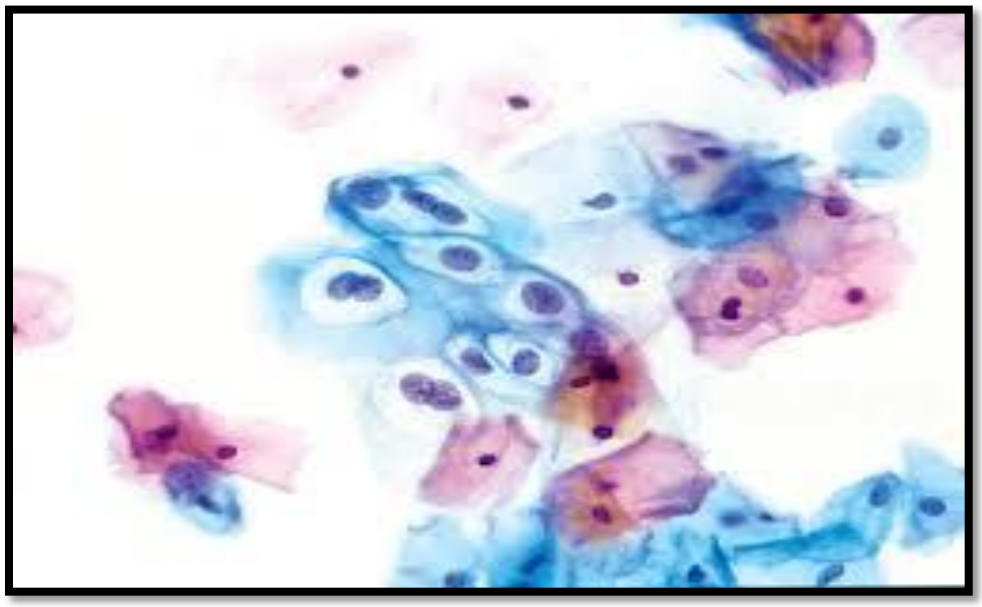


FIG – 13 LBP - ASCUS (High Power View)

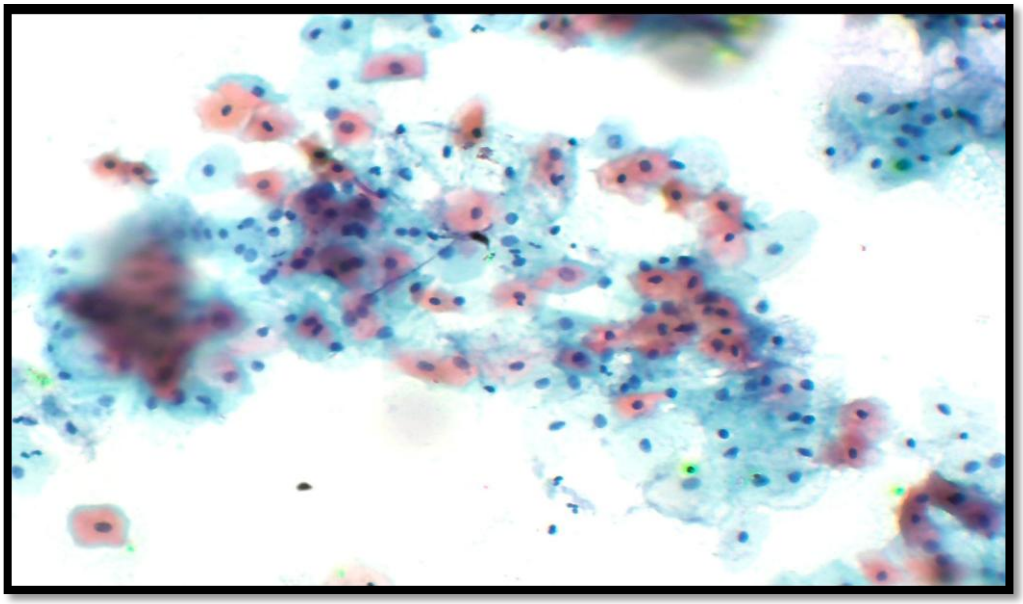


FIG – 14 CONVENTIONAL PAP –CANDIDA (40X)

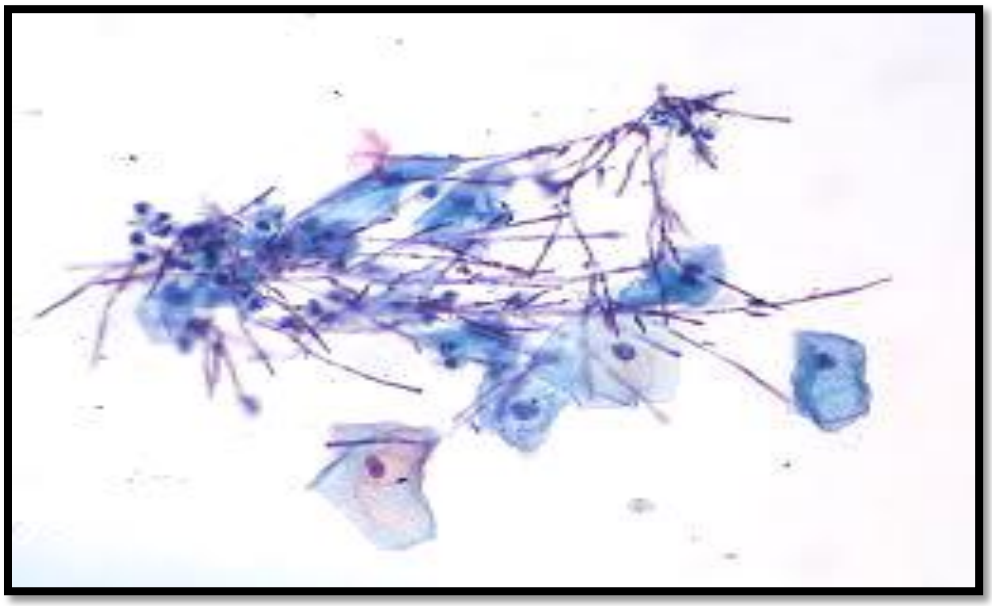


FIG – 15 LBP –CANDIDA (40X)

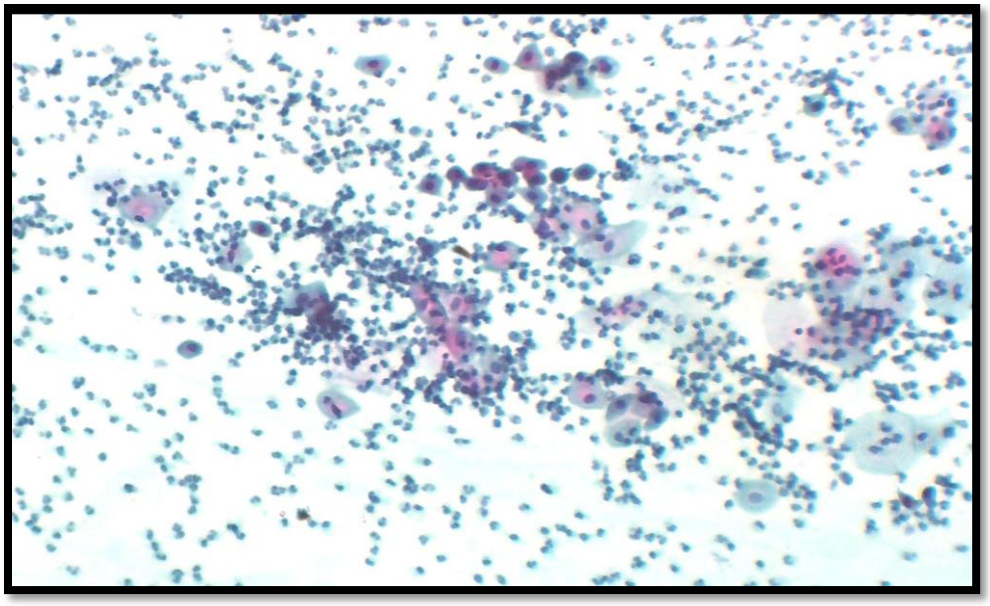


FIG – 16 CONVENTIONAL- ATROPHIC SMEAR (10X)

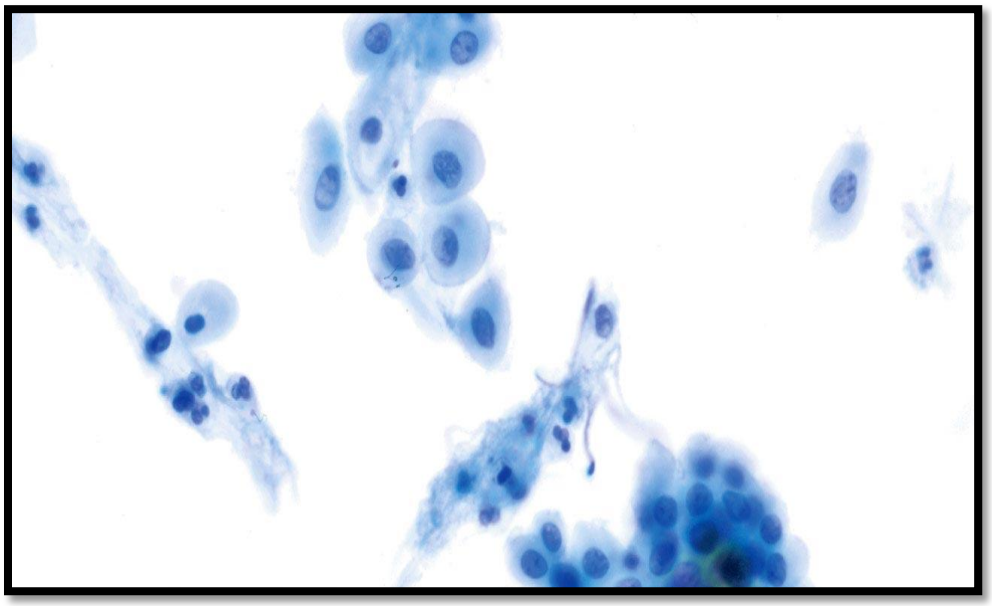


FIG – 17 LBC-ATROPHIC SMEAR (40X)

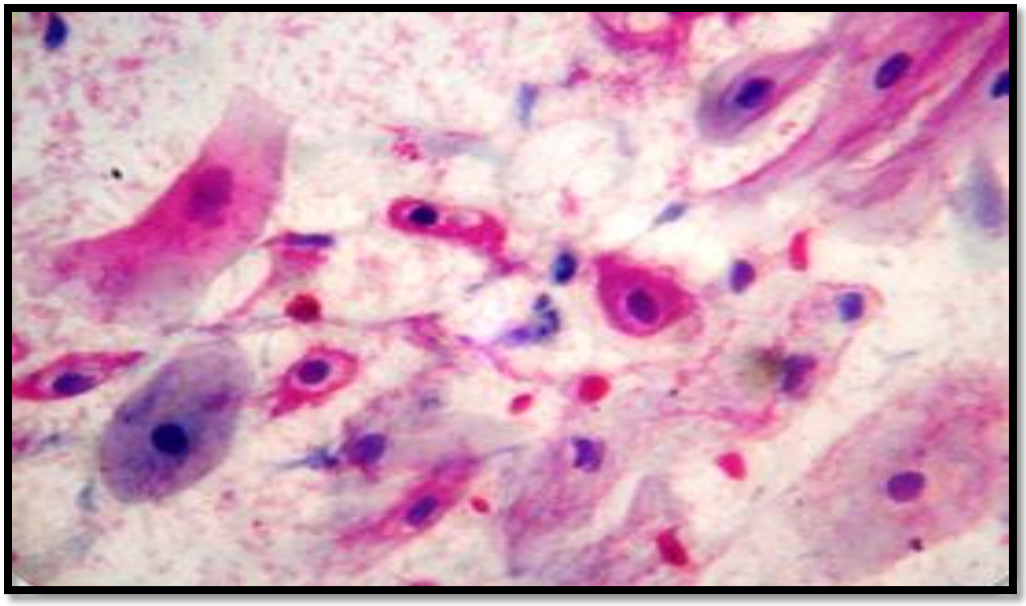


FIG – 18 CONVENTIONAL –LSIL (High power view)

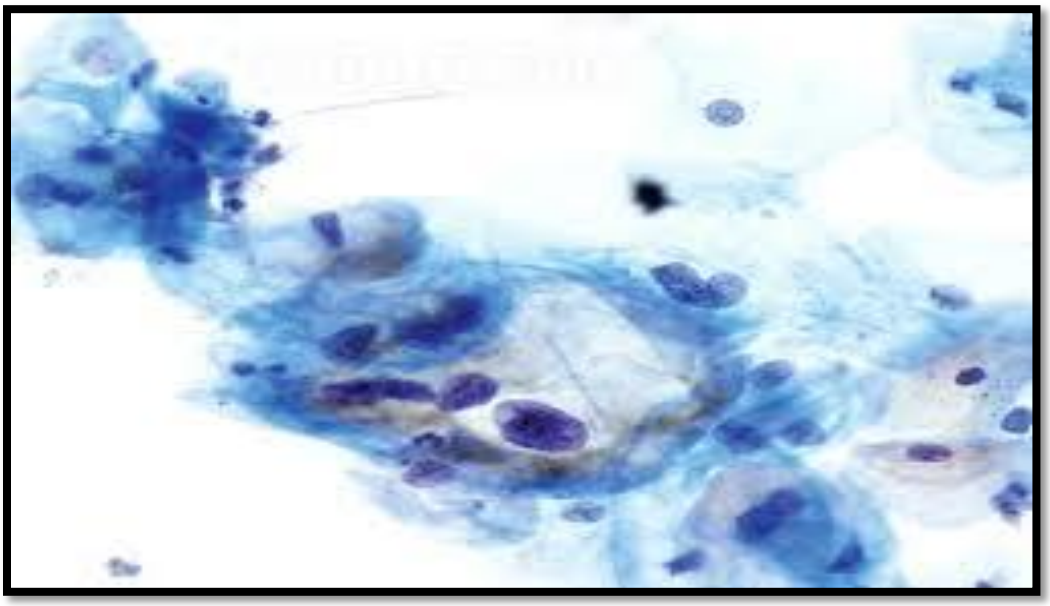


FIG – 19 LBP –LSIL (High power view)

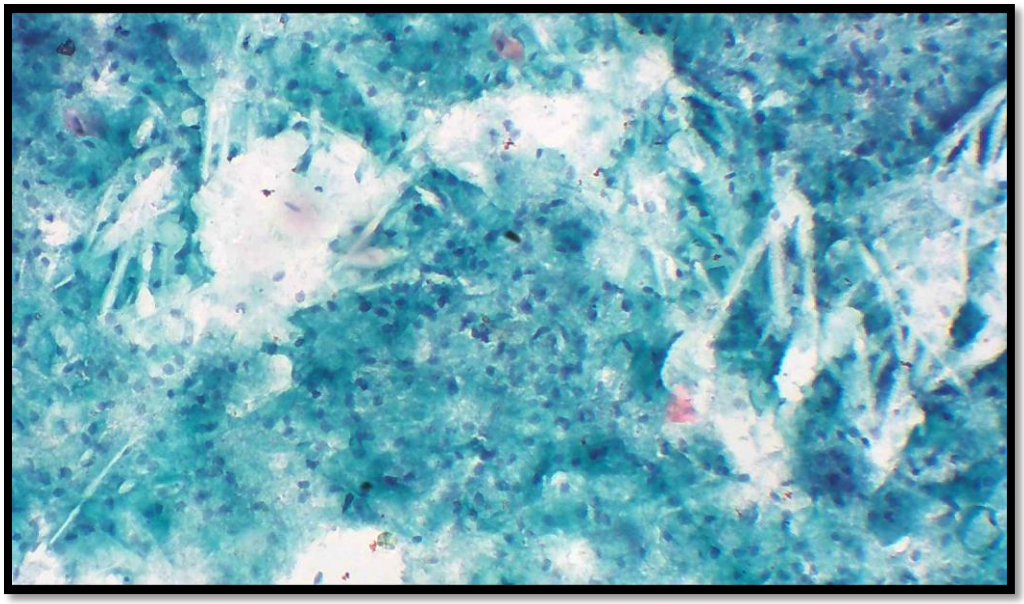


FIG – 20 CONVENTIONAL-CYTOLYSIS(10X)

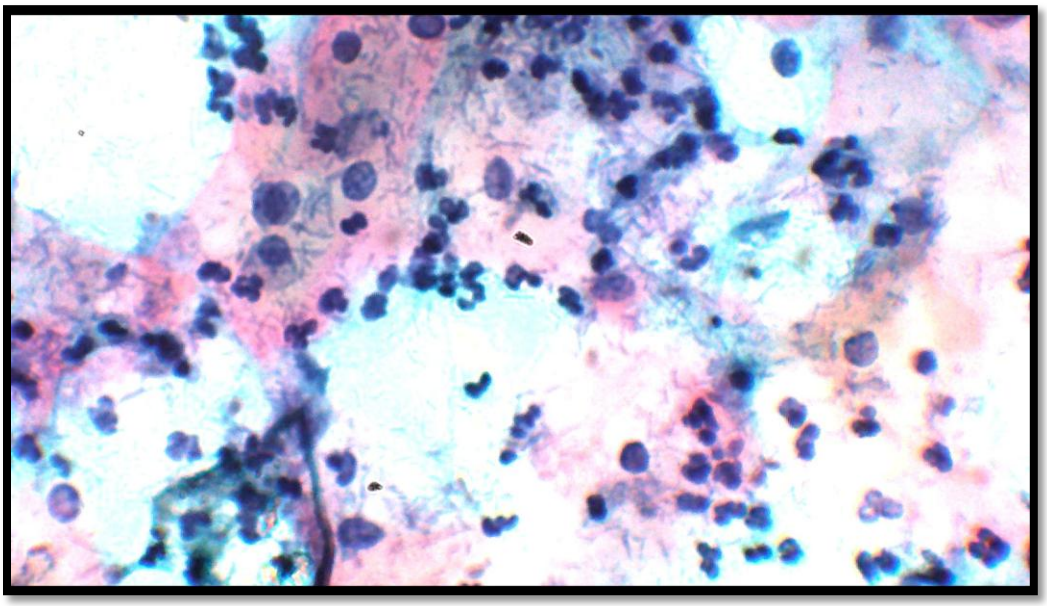


FIG – 21 CONVENTIONAL PAP –LACTOBACILLI (40X)

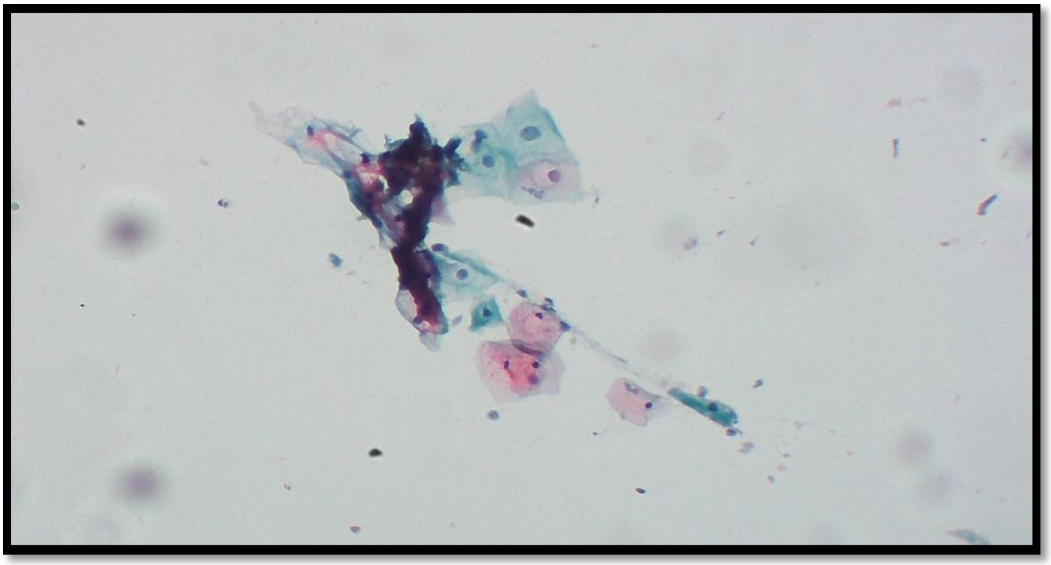


FIG – 22 CONVENTIONAL-KOILOCYTIC ATYPIA(40X)

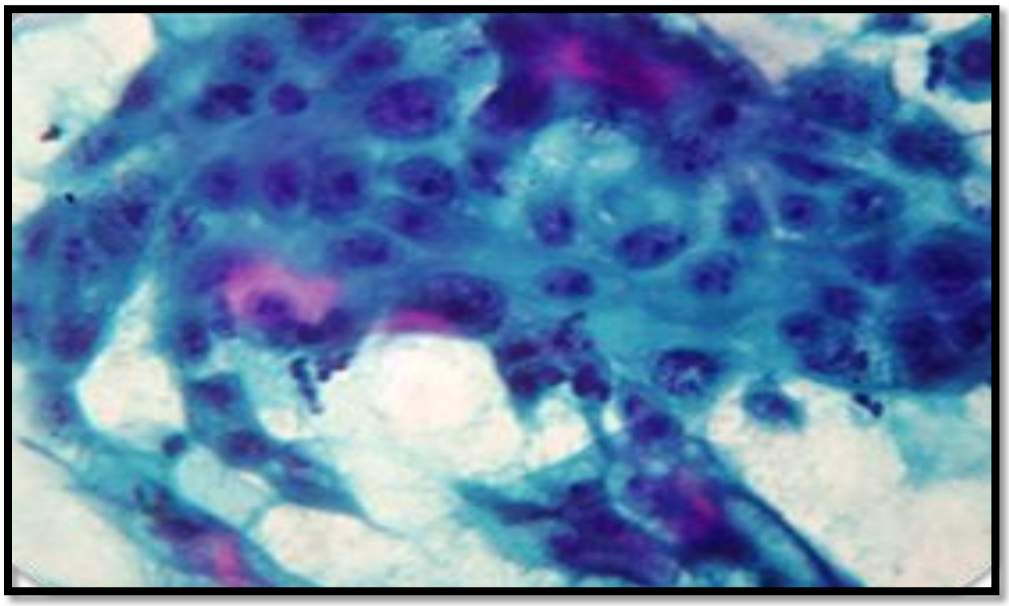


FIG – 23 CONVENTIONAL PAP –HSIL (High power view)

Discussion

The cytological diagnosis of cervical smears has become a very important screening test for the detection of pre invasive and invasive cervical epithelial abnormalities. Screening of female population for cervical neoplasia is simple, inexpensive and reliable method which greatly reduces the mortality and morbidity associated with carcinoma cervix, if detected in its pre invasive stage.

In the present study it has been planned to study the changes in cervical cytology smears and the efficacy of conventional pap smears compared with the liquid based cytology smears on 110 women.

The study group comprises women of age group between 25- 60 years. This age group has been taken because, sexually transmitted infections are more common in this younger age group Thomas et al [64] and 20% of cervical cancers are detected in the peri menopausal groups. So there is a need for screening programmes in this group[110].

In our study in the LBC method it was founded inflammatory smears and ASCUS, LSIL, HSIL were found to be more in the higher age category (> 30 yrs) compared with the lower age category (< 30 yrs) while atrophic lesions were found to be detected more in the lower age group than in the

higher age group. In a study conducted by Ranabhat et al [110] he found that 80% of abnormal epithelial lesions were found in the age group of above 40 years. A study done in India also shows that 51.5% of squamous intraepithelial lesions and 75.3% of cancer cases were detected in the women of above 40 years of age.

Mayavati Mhaskeet et al[111] in their study of risk factor association and cervical dysplasia show the association between early age of marriage and cervical cancer. In our study, most of the women got married between the age of 18-26 years (lower age group upto 23years). Abnormal lesions were detected more or less equal in both age groups.

Another study by Sreejatha Raychaudhiri et al[112] showed that early age of marriage indicated an early exposure to sexual activities and early pregnancy are risk factors for carcinoma cervix.

In this study most of the women got delivered between 18 to 26 years (90%). Inflammatory, ASCUS, LSIL, HSIL were found to be detected more in the women who got delivered in the lower age group. A study done by Misra et al [65], showed the effect of this early consummation is an increase chance of cervical cancer as in case of early marriage. In his study he showed the frequency of SIL was maximum in women who were married

between 21-30 years and carcinoma cervix was maximum in women who married late, after 30 years.

Most of the women in our study who have one to two deliveries at average duration (spacing) between deliveries was 2 years or more. Very high parity was not seen in our study. Multiparity and decreasing spacing between deliveries are risk factors for carcinoma cervix. Study by Misra et al[65], also showed the frequency of both premalignant lesion and cervical cancer is more increasing with parity.

In our study 6.5 % women had a history of usage of Cu-T. Among this one woman has ASCUS in LBC and the number of abnormal lesions is found to be less in women who are using Copper – T and more in women without Copper-T usage. Study done by Lassise et al[113], found a decreased risk with increased duration of Copper- T use. This supports a possible protective effect of copper IUD use on the development of invasive cervical cancer.

In our study with conventional method we have found 5.5% of unsatisfactory smears, 7.3% of normal smears, 80.9% inflammatory smears, 2.7% of atrophic smears, 0.9% of ASCUS, 1.8% LSIL, and 0.9% of HSIL.

In our study of Liquid based Cytology there was no unsatisfactory smears, 8.2% of normal smears, 80% of inflammatory smears, 2.7% of

strophic smears, 4.5% of ASCUS, 3.6% of LSIL, 0.9% of HSIL were detected.

In this study we found the unsatisfactory smears were more in the conventional method, compared to liquid based cytology and also normal smears found to be more in liquid based cytology which was found to be statistically significant with the study done by Chinaka CC et al[114], who also found more unsatisfactory smears with conventional methods than Liquid based cytology. This may be due to the thick smears by obscuring factors like blood, mucus and inflammatory cells in conventional method.

Nandhini N M et al[15], In this study, the number of unsatisfactory found that there are varying unsatisfactory smear rates which is contradictory to our study. In his study the number of unsatisfactory smears by conventional smears was 9 as compared to LBC which has one unsatisfactory smears

In this study the detection of smears negative for intraepithelial lesion are more or less equal in numbers 80% in both conventional and LBC which was the similar result noted in the study conducted by Annie N. Y. chung et al[111].

In this study 90% of all abnormal epithelial lesions were found in the age group of above 40 years. A study already done in our country has found

that 51.5% of squamous intraepithelial lesion cases and 75.3% of cancer cases were detected in women above 40 years by Misra et al[65], in 2009.

In our study 905 of the abnormal epithelial lesions were found in multipara, who has given delivery two or more. In the study by Rhababhat et al[110], 80% of the epithelial abnormalities were found in the age group of more than 40 years.

In this study women who had a history of chronic Vaginal Infection, inflammatory smears, atrophic, ASCUS, LSIL, HSIL, were found to be detected more in higher age group. The p value obtained in statistically significant.

In this study women who had a history of Oral Contraceptive Pills, ASCUS, LSIL, HSIL were found to be less when compares to women without taking Oral contraceptive Pills.

The microscopic detail of infectious agents like candida and bacterial vaginosiss detection was enhanced in LBC compared with conventional method. The results in the current study demonstrate that Thin prep reduces the possibility of unsatisfactory smears. It improves efficiency in detecting LSIL to ASCUS and reduced number of cells reported to have reactive atypia. This is due to better demonstration of koilocytes in reactive atypia. Annie N.Y. Cheung et al[101] in his study more cases of fungal infections were

identified in thin prep. In our study 4 cases of Bacterial vaginosis and 2 cases of candida were detected in both conventional and liquid based cytology smears.

In comparing both studies, the results of the two modalities tent to be in agreement about 90% of the time.

Thin prep calssifies 6.8% of the conventially non neoplatic smears as abnormally neioplastic smears with 0.2% as LSIL and 0.46% as ASCUS. According to Thin prep modality salvages 6.9% of the conventionally prepared smears (considered false negative) which is more or less corresponding to the results of the study conducted by Ovadia Abulafia et al[95],.

In summary thin prep liquid based cytology modality appears more sensitive and specific than conventional smears with increase in detection of LSIL and ASCUS by virtue of getting more satisfactory smears.

This study shows that even through the prevalence of the epithelial lesions is rare, a regular screening programme can find out the early lesions are curable. From our study it is found that premalignant lesions are more detected in LBC, hence the advantage of new technologies should be emphasized. So education and motivation among public as well as the physician community for an emphatic screening programme should be done

as Pap smears is one the simplest test that can diagnose these lesions. The knowledge, attitude and practice (KAP) gap among physician as well as patient community should be adequately addressed.

Conclusion

- After the analysis of 110 cervical smears from women in the age group of 25-60 years, the following conclusions were made.
- The most common finding in cervical smear cytology was inflammatory smears, followed by normal and atrophic smear.
- In this present study it was found that satisfactory smears were significantly more in liquid based cytology compared with conventional smears.
- Though liquid based cytology is more sensitive in detecting the abnormal epithelial lesions, there is no significant statistical difference in my studies.
- It is concluded that thin prep liquid based cytology modality shows more intraepithelial lesions than conventional smears.

Summary

- Duration of the study was from April 2013-March 2014.
- In this period 110 cases are collected and the age group was from 25-60 years.
- All the cases were collected from the obstetrics and gynecology department OPD.
- Most of the participants were in the age group of 41-50yrs were 65(59.1%) women between the age group of 31-45 years were 28(25.5%),women between the age group of 51-60 years were 13(11.8%).
- In our study most of the women had educations up to higher secondary school were 73 (66.4 %). Women had education up to middle school are 14 (12.7%). 10 (9.1%) had education up to primary, 9(8.2%) had education up to degree and the rest 4 (3.6%) had education up to high school.
- In our study most of the women were housewives 76 (69.1%). 18 (16.4%) were employed and women of 16 (14.5%) were cooley.
- In our study, 7(6.4%) women had a history of copper-T use.

- In our study, 13(11.8%) had history of chronic vaginal infection and 97(88.2%) without history of infection.
- In our study, women with history of OCP usage were 4(3.6%) and without usage were 106 (96.4%).
- In our study, among the 110 conventional pap smears, unsatisfactory smears were 6 (5.5%), normal smears were 8 (7.3%), smears characterized as inflammatory were 89 (80.9%), and atrophic smears were 3 (2.7%), smears with features of ASCUS were 1(0.9%), smears with features of LSIL were 2 smears (1.8%),smears with features of HSIL were1 (0.9%)
- In our study among the LBC smears, there were no unsatisfactory smears (0%),normal smears were 9(8.2%),smears characterized as inflammatory were 88(80.0%),atrophic smears were 3(2.7%),smears with features of ASCUS were 5(4.5%),smears with features of LSIL were4(3.6%), and 1(0.9%) smear with features of HSIL were 1(0.9%)
- Lesions detected in the lower age group, normal smears were 3 (9.4%), inflammatory were 25 (78.1%), atrophic smears were 2(6.2%), smears detected as ASCUS were 1(3.1%), smears detected as LSIL were1(3.1%), HSIL were 0(0.0%). Lesions detected in the higher age group, the normal smears were 6(7.7%), inflammatory

were 63 (80.8%), atrophic were 1(1.3%), smears detected as ASCUS were 4(5.1%), LSIL were 3 (3.8%), HSIL were 1 (1.3%). p value obtained is 0.8, it is not significant.

- The unsatisfactory smears were found to be more in conventional and abnormal smears were found to be more in liquid based cytology .
- In summary thin prep liquid based cytology modality appears more sensitive and specific than conventional smears with increase in detection of LSIL and ASCUS by virtue of getting more satisfactory smears.

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Annexures

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Institutional Human Ethics Committee

Ref. No. SMIMS/IHEC/2013/A/06 **Date: 1st July 2013**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2013/A/06,** entitled "Diagnostic Efficacy of Thin Prep Preparation (Liquid Based Cytology) in Comparison to Conventional Pap Smear as a Primary Screening Tool for Cervical Lesions" submitted by Dr. Premalatha A, Postgraduate of Department of Pathology, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 30th of May 2013.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

IHEC Date. 8/7/13

Dr. Rema Menon. N
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CERTIFICATE

We the members of the Research committee have screened the protocol of the dissertation submitted by the P.G. Students Dr. Premalatha. A ..... in detail and found itself to be fit enough for submitting to the IHEC for approval.

Kumari Sheela MS
6.5.2013

Chairperson

Dr. Haneephabi. 6/5/13

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1) Mr. M.B. Kumar - Statistician
2) Dr. Rema Menon - Professor & HOD Pharmacology
3) Dr. Pethuru - Epidemiologist
4) Dr. Kaniraj Peter – Professor of Medicine
5) Dr. Balachandran - Professor of OBG
6) Dr. Sreelal - Professor dental College.

ANNEXURE 1

PAPANICOLAOU STAINING.

S.NO	PROCEDURE	TIME
1	70% Isopropyl alcohol	1 min
2	50% Isopropyl alcohol	1 min
3	Distilled water	1 min
4	Harris hematoxylin	1 min and 30 seconds
5	Tap water	1 min
6	0.5% HCI in 70% isopropyl alcohol	15 seconds
7	Distilled water	1 min
8	Ammoniated alcohol solution.	1 min to 1 ½ min
9	Distilled water	1 min
10	50% isopropyl alcohol	1 min
11	70% isopropyl alcohol	1 min
12	80% isopropyl alcohol	1 min
13	OG solution	2 min, 30 seconds
14	95% isopropyl alcohol	1 min
15	95% isopropyl alcohol	1 min
16	EA 50	2 min
17	95% isopropyl alcohol	1 min

18	95% isopropyl alcohol	1 min
19	Absolute isopropyl alcohol	3 min
20	Absolute isopropyl alcohol	3min
21	Xylene	2 min
22	Xylene	2 min
23	Mount in DPX	

2001 Bethesda System for reporting cervical cytology

General Categorization(Optional)

Negative for intraepithelial lesion or malignancy (NILM)

Epithelial cell abnormality

Other

Interpretation/results

NILM

Organisms

Trichomonas vaginalis

Fungal organisms morphologically consistent with Candida species

Shift in flora suggestive of bacterial vaginosis.

Bacteria morphologically consistent with Actinomyces species

Cellular changes consistent with herpes simplex virus

Other non neoplastic findings(optional to report;list not comprehensive)

Reactive cellular changes associated with;inflammation (includes typical repair); radiation;intrauterine contraceptive device (IUD)

Glandular cells status post hysterectomy

Atrophy

Epithelial cell abnormalities

Squamous cell

Atypical squamous cells(ASC) of undertemined signifance (ASCUS)

Cannot exclude HSIL (ASC-H)

Low grade squamous intraepithelial lesion (LSIL)

High- grade squamous intraepithelial lesion (HSIL)

Squamous cell carcinoma(SCC)

Glandular cell

Atypical glandular cells (AGC); specify endocervical , endometrial ,or not otherwise specified)

Endocervical adenocarcinoma in situ (AIS)

Adenocarcinoma

Other

Endometrial cells in a women older than 40 years of age

Automated Review and Ancillary Testing (Include as appropriate)

Educational Notes and Suggestions (Optional)

The 2001 Bethesda System Categories for Specimen Adequacy

Satisfactory for Evaluation

A satisfactory component must be present. note the presence or absence of endocervical or transformation zone component. Obscuring elements like inflammation, blood,drying artifact, other may be mentioned if 50% to 755 of epithelial cells are obscured.The minimum number of squamous cells for adequacy depends on the preparation method:

Liquid-based:5000 well preserved and well visualized squamous cells. Conventional: 8000 to 12,000 well preserved and well visualized squamous cells.

In the 2001 Bethesda System, the presence or absence of an endocervical or transformation zone component is noted on the report. An endocervica component is considered present if 10 or more endocervical or squamous metaplastic cells either isolated or in groups, are present. Currently,a smear without endocervical cells is not considered unsatisfactory, although the absence of an endocervical or transformation zone component is mentioned as a “quality indicator”. Any specimen with abnormal cell is satisfactory for evaluation.The presence of benign endometrial cells does not make a smear satisfactory.

LIST OF ABBREVIATIONS

AIS - Adenocarcinoma in situ

AGC - Atypical glandular cells

ASC - Atypical squamous cells

ASCUS – Atypical squamous cells of undetermined significance.

ASC-H- Atypical squamous cells cannot exclude high grade squamous intraepithelial lesion.

CIN- Cervical intraepithelial neoplasia

CP- Conventional preparation

HSIL – High Grade squamous intraepithelial Lesions.

HPV- Human papillomavirus

HIV- Human immunodefieency virus

IUD- Intrauterine device

LSIL- Low grade squamous intraepithelial lesion

LBC- Liquid based cytology

LBP- Liquid based preparation

N/C- Nuclear to cytoplasmic ratio

NILM- Negative for intraepithelial lesion or malignancy

SIL- Squamous intraepithelial lesion

TBS- The Bethesda System

SCC – Squamous Cell Carcinomu

CONSENT FORM

PART – II

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware that the results of the study may not be directly beneficial to me but will help in the advancement of medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled " DIAGNOSTIC EFFICACY OF THIN PREP PREPARATION(LIQUID BASED CYTOLOGY) IN COMPARISON TO CONVENTIONAL PAP SMEAR AS A PRIMARY SCREENING TOOL FOR CERVICAL LESIONS."

Serial no/Referance no:

Name : **Address :**

Contact no : **Signature of the participant**

Witness

1.

2.

Date :Place : Kulasekharam

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

KULASEKHARAM – 629 161

Case Record Sheet

STUDY TITLE : DIAGNOSTIC EFFICACY OF THIN PREP PREPARATION (LIQUID BASED CYTOLOGY) IN COMPARISON TO CONVENTIONAL PAP SMEAR AS A PRIMARY SCREENING TOOL FOR CERVICAL LESIONS.

PROFORMA

Name :

Date :

Op No :

Age :

Sex :

Address :

Socio-Economic Status :

Presenting complaints : Leucorrhea /Pv Bleeding /Pv Mass

LMP (Last Menstrual Period) :

LCB (Last Child Birth) :

Martial status:

Menstrual history :

Obstetric history :

H/o contraception	:	
Medical/Surgical History	:	
General examination	:	
Per speculum examination	:	
Per vaginal examination	:	
Investigations	:	Conventional pap smear, liquid based cytology smear

Master chart

MASTER CHART															
S.NO	AGE	EDUCATION	OCCUPATION	AGE.AT. MARRIAGE	NO.OF.DELIVERIES	AGE.AT.FIRST.DELIVERY	GAP.d\DELIVERIES	HO.CuT	HO.STD.HIV.PATIENT	HO.STD.HIV.PARTNER	HO.CHR.VAG.INFCETIONS	HO.OCP	CONVENTIONAL	LBC	INFLAM.CATEGORY
1	47	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	1.Yes	2.No	3. Inflammatory	3. Inflammatory	NA
2	50	4. HSS	1. Housewife	28	3.Two	29	2	2. No	No	No	2.No	2.No	6. LSIL	6. LSIL	
3	45	4. HSS	1. Housewife	24	4.Three	25	2	2. No	No	No	2.No	2.No	5. ASCUS	5. ASCUS	
4	39	4. HSS	1. Housewife	24	3.Two	25	3	2. No	No	No	1.Yes	1.Yes	3. Inflammatory	3. Inflammatory	NA
5	33	5. DEGREE	3. Employed	25	3.Two	26	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
6	60	4. HSS	1. Housewife	24	5. Four	26	2	2. No	No	No	1.Yes	1.Yes	3. Inflammatory	3. Inflammatory	NA
7	33	4. HSS	1. Housewife	26	3.Two	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
8	45	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	2.No	2.No	6. LSIL	6. LSIL	
9	25	5. DEGREE	3. Employed	21	2.One	23		2. No	No	No	1.Yes	2.No	2. Normal	2. Normal	
10	50	2. MIDDLE SCHOOL	1. Housewife	22	4.Three	23	2	2. No	No	No	1.Yes	1.Yes	2. Normal	2. Normal	
11	45	4. HSS	1. Housewife	25	3.Two	26	2	2. No	No	No	1.Yes	2.No	2. Normal	2. Normal	
12	49	4. HSS	1. Housewife	24	5. Four	25	2	2. No	No	No	1.Yes	2.No	2. Normal	2. Normal	
13	47	3. HIGH SCHOOL	1. Housewife	25	5. Four	26	2	2. No	No	No	1.Yes	1.Yes	3. Inflammatory	3. Inflammatory	NA
14	41	4. HSS	1. Housewife	25	2.One	26		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
15	42	4. HSS	1. Housewife	26	4.Three	27	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
16	40	5. DEGREE	3. Employed	25	4.Three	26	2	2. No	No	No	1.Yes	2.No	3. Inflammatory	3. Inflammatory	NA
17	43	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	1.Yes	2.No	3. Inflammatory	3. Inflammatory	NA
18	40	5. DEGREE	3. Employed	24	4.Three	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
19	40	4. HSS	1. Housewife	24	3.Two	25	1	2. No	No	No	1.Yes	2.No	3. Inflammatory	3. Inflammatory	NA
20	44	4. HSS	1. Housewife	23	2.One	24		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
21	35	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
22	37	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
23	27	5. DEGREE	3. Employed	22	2.One	23		2. No	No	No	1.Yes	2.No	3. Inflammatory	3. Inflammatory	NA
24	35	4. HSS	1. Housewife	26	3.Two	27	2	2. No	No	No	2.No	2.No	2. Normal	2. Normal	
25	40	4. HSS	1. Housewife	24	4.Three	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA

S.NO	AGE	EDUCATION	OCCUPATION	AGE.AT. MARRIAGE	NO.OF.DELIVERIES	AGE.AT.FIRST.DELIVERY	GAP.d\DELIVERIES	HO.CuT	HO.STD.HIV.PATIENT	HO.STD.HIV.PARTNER	HO.CHR.VAG.INFCETION	HO.OCP	CONVENTIONAL	LBC	INFLAM.CATEGORY
26	36	4. HSS	1. Housewife	23	4.Three	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
27	30	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
28	53	4. HSS	1. Housewife	25	5. Four	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Bacteria
29	31	5. DEGREE	3. Employed	24	2.One	26		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
30	43	3. HIGH SCHOOL	1. Housewife	22	3.Two	23	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Bacteria
31	43	2. MIDDLE SCHOOL	2. Cooly	26	5. Four	27	3	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
32	28	5. DEGREE	3. Employed	21	2.One	23		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
33	46	4. HSS	1. Housewife	22	3.Two	24	4	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Candida
34	34	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	2. Normal	2. Normal	
35	37	2. MIDDLE SCHOOL	2. Cooly	21	4.Three	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Bacteria
36	36	4. HSS	1. Housewife	18	3.Two	21	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
37	39	4. HSS	1. Housewife	22	3.Two	23	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
38	45	4. HSS	1. Housewife	23	3.Two	24	3	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
39	35	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	6. LSIL	
40	38	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
41	41	4. HSS	1. Housewife	23	3.Two	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
42	43	1. PRIMARY	2. Cooly	22	4.Three	23	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Candida
43	56	2. MIDDLE SCHOOL	1. Housewife	26	4.Three	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
44	42	4. HSS	1. Housewife	25	3.Two	26	2	2. No	No	No	2.No	2.No	2. Normal	2. Normal	
45	41	4. HSS	1. Housewife	26	3.Two	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	5. ASCUS	
46	50	4. HSS	1. Housewife	26	5. Four	28	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
47	43	2. MIDDLE SCHOOL	2. Cooly	26	4.Three	28	2	2. No	No	No	2.No	2.No	2. Normal	2. Normal	
48	53	2. MIDDLE SCHOOL	3. Employed	26	4.Three	28	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
49	47	4. HSS	1. Housewife	27	4.Three	29	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
50	50	4. HSS	1. Housewife	26	4.Three	28	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
51	44	4. HSS	3. Employed	26	3.Two	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA

S.NO	AGE	EDUCATION	OCCUPATION	AGE.AT. MARRIAGE	NO.OF.DELIVERIES	AGE.AT.FIRST.DELIVERY	GAP.d\DELIVERIES	HO.CuT	HO.STD.HIV.PATIENT	HO.STD.HIV.PARTNER	HO.CHR.VAG.INFCETIONS	HO.OCP	CONVENTIONAL	LBC	INFLAM.CATEGORY
52	42	4. HSS	1. Housewife	26	3.Two	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
53	42	4. HSS	1. Housewife	24	3.Two	26	1	2. No	No	No	2.No	2.No	2. Normal	2. Normal	
54	49	1. PRIMARY	2. Cooly	26	3.Two	28	1	1.Yes	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
55	41	2. MIDDLE SCHOOL	1. Housewife	26	2.One	28		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
56	46	2. MIDDLE SCHOOL	1. Housewife	28	3.Two	30	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
57	41	5. DEGREE	3. Employed	27	2.One	28		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
58	42	2. MIDDLE SCHOOL	1. Housewife	26	2.One	27		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
59	42	4. HSS	2. Cooly	22	2.One	24		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
60	50	1. PRIMARY	1. Housewife	24	6. Five	25	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
61	49	1. PRIMARY	1. Housewife	27	3.Two	28	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
62	47	4. HSS	3. Employed	25	5. Four	26	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
63	52	4. HSS	2. Cooly	22	1.Nil			2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
64	54	4. HSS	1. Housewife	24	2.One	26		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
65	42	4. HSS	1. Housewife	17	2.One	20		2. No	No	No	2.No	2.No	3. Inflammatory	6. LSIL	
66	47	2. MIDDLE SCHOOL	2. Cooly	21	3.Two	23	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
67	40	4. HSS	1. Housewife	24	3.Two	26	2	2. No	No	No	2.No	2.No	4. Atrophic	4. Atrophic	
68	53	1. PRIMARY	2. Cooly	24	4.Three	26	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
69	42	5. DEGREE	3. Employed	24	2.One	26		1.Yes	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
70	46	4. HSS	1. Housewife	24	1.Nil			2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
71	48	4. HSS	1. Housewife	23	3.Two	26	4	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
72	46	4. HSS	3. Employed	25	3.Two	27	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
73	48	1. PRIMARY	1. Housewife	23	3.Two	25	2	1.Yes	No	No	1.Yes	2.No	3. Inflammatory	5. ASCUS	
74	54	1. PRIMARY	1. Housewife	21	3.Two	23	2	2. No	No	No	2.No	2.No	4. Atrophic	4. Atrophic	
75	42	4. HSS	1. Housewife	22	3.Two	26	2	1.Yes	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Bacteria
76	50	4. HSS	1. Housewife	26	3.Two	28	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Reactive
77	44	4. HSS	3. Employed	20	3.Two	22	3	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
78	55	1. PRIMARY	2. Cooly	26	4.Three	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
79	51	2. MIDDLE SCHOOL	2. Cooly	22	1.Nil			2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA

80	41	2. MIDDLE SCHOOL	1. Housewife	22	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	Reactive
81	45	2. MIDDLE SCHOOL	2. Cooly	20	2.One	22		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
82	52	4. HSS	1. Housewife	28	3.Two	29	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
83	47	3. HIGH SCHOOL	3. Employed	26	2.One	27		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
84	47	4. HSS	2. Cooly	26	1.Nil			2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
85	54	1. PRIMARY	2. Cooly	17	4.Three	19	1	2. No	No	No	2.No	2.No	3. Inflammatory	5. ASCUS	
86	48	3. HIGH SCHOOL	1. Housewife	24	2.One	26		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
87	48	4. HSS	1. Housewife	24	3.Two	26	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
88	44	4. HSS	1. Housewife	26	4.Three	27	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
89	43	4. HSS	3. Employed	22	1.Nil			2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
90	44	4. HSS	3. Employed	26	3.Two	28	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
91	46	4. HSS	1. Housewife	26	3.Two	28	2	1.Yes	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
92	55	1. PRIMARY	2. Cooly	24	4.Three	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
93	47	4. HSS	1. Housewife	20	4.Three	22	2	1.Yes	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
94	43	4. HSS	3. Employed	22	3.Two	24	1	1.Yes	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
95	49	4. HSS	1. Housewife	22	3.Two	24	3	2. No	No	No	2.No	2.No	7. HSIL	7. HSIL	
96	36	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
97	39	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
98	42	4. HSS	1. Housewife	22	3.Two	23	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
99	46	4. HSS	1. Housewife	24	4.Three	25	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
100	37	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
101	40	4. HSS	1. Housewife	24	2.One	27		2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
102	40	4. HSS	1. Housewife	25	3.Two	26	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
103	47	2. MIDDLE SCHOOL	2. Cooly	20	1.Nil			2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
104	43	4. HSS	1. Housewife	24	4.Three	25	1	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
105	31	4. HSS	1. Housewife	25	3.Two	26	1	2. No	No	No	2.No	2.No	4. Atrophic	4. Atrophic	
106	41	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
107	40	4. HSS	1. Housewife	24	3.Two	25	2	2. No	No	No	2.No	2.No	3. Inflammatory	5. ASCUS	
108	45	4. HSS	1. Housewife	26	3.Two	27	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
109	36	4. HSS	1. Housewife	23	3.Two	24	2	2. No	No	No	2.No	2.No	3. Inflammatory	3. Inflammatory	NA
110	40	4. HSS	1. Housewife	22	3.Two	24	2	2. No	No	No	2.No	2.No	1.Unsatisfactory	3. Inflammatory	NA